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Date: December 16, 1999

Docket No.: 2121-154P

Honorable Commissioner of Patents
Washington, D.C. 20231

Sir:

This is a Request for filing a ____ continuation X divisional application under 37 C.F.R. § 1.53(b) of pending prior application Serial No. 08/817,188 filed on May 15, 1997

by
Marc DeBlock

for
Genetic Transformation Using a PARP Inhibitor

1. X Enclosed is a true copy of the complete prior above-identified application as filed, consisting of specification, claims and declaration.

2. X The filing fee has been calculated as follows:

			LARGE ENTITY	SMALL ENTITY
	BASIC FEE		$760.00	$380.00
	NUMBER FILED	NUMBER EXTRA	RATE FEE	RATE FEE
TOTAL CLAIMS	13- 20 =	0	x 18 = $	x 9 = $
INDEPENDENT CLAIMS	1- 3 =	0	x 78 = $	x 39 = $
MULTIPLE DEPENDENT CLAIMS PRESENTED			+ $260.00	+ $135.00
		TOTAL	$760.00	

3. √X A check in the amount of $ 760.00 to cover the filing fee and recording fee (if applicable) is enclosed.

4. ___ Please charge Deposit Account No. 02-2448 in the amount of $_____. A triplicate copy of this request is enclosed.

5. X Amend the specification by inserting before the first line thereof the following:

--This application is a ___ continuation X divisional of copending application Serial No. 08/817,188, filed on May 15, 1997, Application Serial No. 08/817,188 is a 371 national application of international application PCT/EP96/03366, filed on July 31, 1996. The entire contents of these applications are hereby incorporated by reference.--

6. ___ Transfer the drawings from the prior application to this application and abandon said prior application as of the filing date accorded this application. A duplicate copy of this request is enclosed for filing in the prior application file.

7. ___ Enclosed is/are _____ sheet(s) of _____ drawings.

8. ___ A verified statement claiming small entity status was

filed in prior application Serial No. _____ on _____. See attached copy of verified statement claiming small entity.

9. X The prior application is assigned to Plant Genetic Systems, N.V.

10. ✓X A Preliminary Amendment is enclosed.

11a. ___ Priority of Application No(s). _____ filed in _____ on _____ is/are claimed under 35 U.S.C. § 119. See attached copy of the Letter claiming priority filed in the prior application on _____.

11b. X Priority of International Appln. PCT/EP96/03366 filed on July 31, 1996 under the Patent Cooperation Treaty and European Application No. 95401844.6 filed in Europe on August 4, 1995 under 35 U.S.C. § 119 are hereby reclaimed.

12. ✓X An Information Disclosure Statement and PTO-1449 form(s) are attached hereto for the Examiner's consideration.

13. X Address all future communications to:

BIRCH, STEWART, KOLASCH & BIRCH, LLP
P.O. Box 747
Falls Church, Virginia 22040-0747
Telephone: (703) 205-8000
or
Customer No. 2292

14. ___ An extension of time for _____ month(s) until _____ has been submitted in parent application Serial No. _____ in order to establish copendency with the present application.

15. ___ Also enclosed herewith is the following:

_____

_____

_____

If necessary, the Commissioner is hereby authorized to charge payment of the following fees associated with this communication or credit any overpayment to Deposit Account No. 02-2448, including any additional filing fee required under 37 C.F.R. § 1.16 or any patent application processing fee under 37 C.F.R. § 1.17.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____

Gerald M. Murphy, Jr.
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GMM/MAA/bsh

PATENT
2121-154P

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicant:	Marc DeBlock		
Serial No:	NEW-Rule 53(b) Div. of 08/817,188	Group:	Unassigned
Filed:	December 16, 1999	Examiner:	Unassigned
For:	GENETIC TRANSFORMATION USING A PARP INHIBITOR		

PRELIMINARY AMENDMENT

Assistant Commissioner of Patent
Washington, D.C. 20231

December 16, 1999

Sir:

The following preliminary amendments and remarks are respectfully submitted in connection with the above-identified application.

IN THE ABSTRACT:

Please add the attached Abstract to the end of the application now on file.

IN THE SPECIFICATION:

Please amend the specification as follows:

Please delete pages 39-60 of the specification containing the sequence listing. Please renumber the remaining pages of the specification, beginning with the claims, consecutively from page 39 of the specification. Please insert the Substitute Sequence

Listing enclosed herewith immediately after the Abstract.

At the top of page 61, please delete "CLAIMS" and substitute --What is claimed is--

IN THE CLAIMS:

Please cancel claims 1-23 without prejudice and without disclaimer of the subject matter contained therein.

Please add the following claims:

--24. A method for assessing the agronomical fitness of a plant or plant material comprising the steps of:

a) subjecting an explant of said plant or plant material to a stress condition;

b) measuring the electron flow in the mitochondrial electron transport chain to assess agronomical fitness in cells of said explant of said plant or said plant material;

c) comparing said measurement to that of explants of control plants or control plant material, under similar conditions as for said explants of said plant or plant material, wherein the greater the amount of electron flow the fitter said plant or plant material.--

--25. The method of Claim 24, wherein said electron flow in the mitochondrial electron transport chain is determined by measuring the capacity of said explant subjected to said stress condition to reduce 2,3,5-triphenyltetrazolium chloride.--

--26. The method of Claim 24, wherein the electron flow in the mitochondrial electron transport chain is determined by measuring the capacity of said explant subjected to said stress condition to reduce 3-(4,5-dimethylthiazol-2-yl)-2,3 diphenyl-2H-tetrazolium.--

--27. The method of Claim 24, wherein said stress condition is selected from salt stress, osmotic stress, stress by incubation in the presence of an inhibitor of poly-ADP-ribose polymerase, stress from extreme temperatures, stress by treatment with sublethal doses of chemicals, stress by treatment with sublethal doses of herbicides, stress by treatment with sublethal doses of heavy metals and stress by irradiation with ultraviolet light.--

--28. The method of Claim 24, wherein said stress condition is salt stress.--

--29. The method of Claim 28, wherein said salt stress is induced by incubation in K-phosphate buffer comprising between 10mM and 80 mM K-phosphate.--

--30. The method of Claim 24, wherein said stress condition is osmotic stress.--

--31. The method of Claim 30, wherein said osmotic stress is induced by incubation in a buffer comprising about 2% sucrose.--

--32. The method of Claim 24, wherein said stress condition is incubation in the presence of an inhibitor of poly-ADP-ribose polymerase.--

--33. The method of Claim 32, wherein said inhibitor of poly-ADP-ribose polymerase is selected from niacinamide, picolinamide, 5-methyl nicotinamide, methylxanthine, thymidine, benzamide, 3-methoxybenzamide, 3-aminobenzamide, 2-aminobenzamide, pyrazinamide, theobromie and theophylline.--

--34. The method of Claim 32, wherein said inhibitor is present in a concentration of from about 100 mg/L to about 1,000 mg/L.--

--35. The method of Claim 24, wherein said explant is selected from callus, hypocotyl explants, shoots, leaf disks and whole leaves.--

--36. The method of Claim 24, wherein said plant or plant material is a transenic plant or transgenic plant material.--

REMARKS

Claims 24-36 are now pending in this application.

Support for new claims 24-36 may be found on pages 15-23 of the specification. Additional support for claims 33 and 34 may be found on pages 6 and 8, respectively.

The present application is a divisional of parent application Serial No. 08/817,188, filed May 15, 1997, which is filed to pursue subject matter not covered or specifically claimed in the allowed claims of the parent application.

In fulfillment of the requirements under 37 C.F.R.§§1.821-1.825 applicants respectfully request that the disk copy of the sequence listing submitted on July 22, 1999, as file 2121-127P.sub, in parent application No. 08/817,188, be transferred to the present application.

Favorable action and early allowance of the claims are

respectfully requested.

If the Examiner has any questions concerning this application, he is requested to contact the undersigned at (703) 205-8000 in the Washington, D.C. area.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By _____
Gerald M. Murphy, Jr.
Reg. No. 28,977

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GMM/MAA/bsh
enclosure

ABSTRACT OF THE DISCLOSURE

The invention concerns a process for producing transgenic plant cells, which comprises: contacting a culture of plant cells with an inhibitor of poly-(ADP-ribose) polymerase, prior to transformation, for a period of time sufficient to reduce the response of the cultured cells to stress and to reduce their metabolism. The untransformed cells are then contacted with foreign DNA comprising at least one gene of interest under conditions in which the foreign DNA is taken up by the untransformed cells and the gene of interest is stably integrated in the nuclear genome of the untransformed cells to produce the transgenic cells. The transgenic plant cells are recovered from the culture. The invention further concerns a process for increasing the frequency of obtaining transgenic plant cells, via Agrobacterium-mediated transformation, which comprises: contacting a culture of plant cells with an inhibitor or poly(ADP-ribose) polymerase prior to transformation for a period of approximately 1 to 2 days or culturing transgenic plant cells after transformation in a medium containing an inhibitor of poly(ADP-ribose)polymerase for a period of time of approximately 1 to 14 days.

GENETIC TRANSFORMATION USING A PARP INHIBITOR

This invention is related to tissue culture of eucaryotic cells and improved techniques to obtain genetically transformed eucaryotic cells and organisms, such as transgenic plant cells or plants, by lowering the stress reaction of cultured eucaryotic cells prior to contacting the cells with foreign DNA, particularly by specific inhibition of poly-(ADP-ribose) polymerase.

Background to the invention

Over the years many techniques for the genetic transformation of higher organisms (animals and plants) have been developed. In these techniques it is the ultimate goal to obtain a transgenic organism, e.g. a plant, in which all cells contain a foreign DNA comprising a gene of interest (the so-called transgene) stably integrated in their genome, particularly their nuclear genome.

Transformation is a complex process which always involves the contacting of starting cells with a DNA, usually a DNA comprising foreign gene(s) of interest. The contacting of the cells with the DNA is carried out under conditions that promote the uptake of the DNA by the cells and the integration of the DNA, including the gene(s) of interest into the genome of the cell.

Starting cells for transformation are usually cells that have been cultured in vitro for some time. After contacting the cells with the DNA, the transformed cells generally need to be cultured in vitro for a certain period in order to separate the transformed cells from the non-transformed cells and, in the case of plants, to regenerate transformed plants from the transformed cells. Indeed, complete plants can be regenerated from individual transformed cells thus ensuring that all cells of the regenerated plant will contain the transgene.

In many plants, genetic transformation can be achieved by using the natural capacity of certain Agrobacterium strains to introduce a part of their Ti-plasmid, i.e. the T-DNA, into plant cells and to integrate this T-DNA into the nuclear genome of the cells. It was found that the part of the Ti-plasmid that is transferred and integrated is delineated by specific DNA sequences, the so-called left and right T-DNA border sequences and that the natural T-DNA sequences between these border sequences can be replaced by foreign DNA (European Patent Publication "EP" 116718; Deblaere et al, 1987 Meth.Enzymol. 153:277-293).

Certain plant species have proven to be recalcitrant to Agrobacterium mediated transformation and in these species, as well as in animals, genetic transformation has been achieved by means of direct gene transfer by which DNA is inserted into the cells by physical and/or chemical means, such as by electroporation, by treatment of the cells with polyethyleneglycol (PEG), by bombardment of the cells with DNA-coated microprojectiles, etc. (WO 92/09696; Potrykus et al, 1991, Annu.Rev.Plant Physiol.Plant Mol.Biol. 42:205-225).

Genetic transformation of eucaryotic cells is generally a random event, i.e. the transgene is integrated in the genome at random positions. Often several copies (or parts of copies) of the transforming DNA are integrated in a single position, and/or at different positions, resulting in a transformed cell containing multiple copies of the transgene.

The expression of the transgene is known to be influenced by its position in the genome. For instance, a foreign DNA, when introduced in a plant cell appears to integrate randomly in the plant genome. Examination of independently transformed plants has shown a high degree of variability (up to 100-fold) in the expression level of the introduced gene. Several studies have shown no correlation between this "between-transformant variability" and the copy

number of the introduced DNA at a given locus. It has been suggested that some of the variability in expression of introduced genes in transgenic plants is a consequence of "position effects" caused by influences of adjacent plant genomic DNA. Other factors that could contribute to the variability in expression are physiological variability of the plant material, differences in the number of independent T-DNA loci in different transformants or the inhibitory effects of certain T-DNA structures on gene expression. Between-transformant variability in expression has been observed for the majority of introduced genes in transgenic plants. The variability in expression of many introduced genes in independent transgenic plants necessitates large numbers of transgenic plants to be assayed to accurately quantitate the expression of the gene. It would be of great importance if the amount of between-transformant variability could be reduced (Dean et al, 1988, NAR 16:9267-9283).

If the transgene is under the control of a tissue-specific promoter, with the expectation that it will be expressed in selected tissues of the transformed organisms, the position effects can lead, at least in some transformants, to loss of specificity of the promoter and expression of the transgene in undesired tissues, e.g. in tissue cultured in vitro.

Factors that are known to influence the efficiency and quality of the genetic transformation process are the method of DNA delivery, specific tissue culture conditions, the physiological and metabolic state of the target cells etc. Direct gene transfer methods for instance are generally known to result in transformed organisms with a high copy number of the transgene.
Many of these factors are not under the control of man.

Summary of the Invention

This invention provides a process for producing transgenic eucaryotic cells, particularly plant cells. The process comprises contacting a culture of untransformed cells with an inhibitor of poly-(ADP-ribose) for a period of time sufficient to reduce the response of the cultured cells to stress and to reduce the metabolism of the cultured cells, particularly to reduce the electron flow in the mitochondrial electron transport chain. The untransformed cells are then contacted with foreign DNA comprising at least one gene of interest under conditions in which the foreign DNA is taken up by the untransformed cells and the gene of interest is stably integrated in the nuclear genome of the untransformed cells to produce the transgenic cells which are recovered from the culture.

The process may preferably comprise contacting untransformed eucaryotic (e.g.) cells with foreign DNA comprising at least one gene of interest under conditions in which the foreign DNA is taken up by the untransformed cells and the gene of interest is stably integrated in the nuclear genome of the untransformed cells to produce the transgenic cells. The untransformed cells are cultured in vitro in a culture medium containing an inhibitor of poly-(ADP-ribose) polymerase, preferably niacinamide, preferably for at least 2 to 3 days, particularly for at least 4 days (e.g. 4-5 days), before the contacting of the untransformed cells with the foreign DNA. The inhibitor can in addition also be applied to cultured cells that are being contacted or that have been contacted the foreign DNA.

Description of the Invention

The present invention is based on the observations that poly-(ADP-ribose) polymerase (PARP) is an enzyme that is involved in regulating the general metabolic state of an eucaryotic cell and that inhibition of this enzyme can be

used to influence the metabolic state of cells which are targeted for transformation (or which are being transformed) to increase the efficiency and/or quality of transformation.

In mammalians, PARP is a monomeric nuclear Zn-finger protein of about 116 kD that is closely associated with nuclear DNA, particularly with actively transcribed euchromatic regions (Shah et al, 1995, Anal.Biochem. 227:1-13). The protein is normally an inactive enzyme but is known to be activated by nicked or otherwise damaged DNA. Active PARP transfers the ADP-ribose moiety of NAD+ to various nuclear proteins to synthesize a polymer of ADP-ribose bound to these proteins which include PARP itself, polymerases, histones, endonuclease etc. The proteins on which such a ADP-ribose polymer is synthesized become biologically inactive (de Murcia et al, 1994, TIBS 19:172-176; Cleaver et al, 1991, Mutation Res. 257:1-18).

The biological function of PARP is largely unknown but the enzyme has been implicated in :

- enhancement of DNA repair (Satoh et al, 1992, Nature 356:356-358; Satoh et al, 1993, J.Biol.Chem. 268:5480-5487),
- recombination events : in general inhibition of PARP is observed to inhibit illegitimate recombination and to increase intrachromosomal recombination but it does apparently not affect extrachromosomal recombination (Farzaneh et al, 1988, NAR 16:11319-11326; Waldman and Waldman, 1990, NAR 18:5981-5988; Waldman and Waldman, 1991, NAR 19:5943-5947),
- regulation of gene expression : inhibition of PARP is observed to decrease gene expression (Girod et al, 1991,Plant Cell, Tissue and Organ Culture 25:1-12);
- reducing the amount of available NAD+ (and by consequence its precursor ATP) : this results in a general slowing down of cell metabolism (Lazebnik

et al, 1994, 371:346-347; Gaal et al, 1987, TIBS 12:129-130; Cleaver et al, supra)

It is known that PARP can be efficiently inhibited by a number of compounds (Durkacz et al, 1980, Nature 283:593-596; Sims et al, 1982, Biochemistry 21:1813-1821). Examples of such compounds are certain pyridine analogs such as nicotinamide analoques, including niacinamide, picolinamide, and 5-methyl nicotinamide; purine analogs like methylxanthines; thymidine; pyrazinamide analogs and many aromatic amides such as many benzamide analogs including benzamide, 3-methoxybenzamide and 3-aminobenzamide. For the purpose of this invention a PARP inhibitor is generally understood as any specific inhibitor of poly-(ADP-ribose) polymerase which can be taken up by a eucaryotic cell, particularly a plant cell, and which has a inhibition constant (Ki) which is lower than $1x10^{-5}$, particularly lower than $1x10^{-6}$. Generally it is desired that the PARP inhibitor used with this invention be a compound which in human lymphocytes, cultured in medium containing the inhibitor at a concentration of 2 mM, results in a 80-90 % inhibition of PARP (Sims et al, supra). Generally it is also preferred that cells cultured in medium containing the PARP inhibitor retain their capacity of DNA repair.
Particularly preferred PARP inhibitors are those listed above and especially niacinamide (nicotinamide), picolinamide, 5-methylnicotinamide, 2-aminobenzamide, pyrazinamide, theobromine and theophylline. Particularly niacinamide is believed to be a useful inhibitor for the purpose of this invention.

Basically the present invention provides a modification of existing procedures for the genetic transformation of eucaryotic cells, particularly plant cells, by including in the medium in which such cells are cultured a PARP inhibitor such as niacinamide, for a defined period of time. In particular the PARP inhibitor is added to the culture medium at least 1 day prior to the moment (the "contacting

time") at which the cells are contacted with foreign DNA comprising one or more genes of interest. However, depending on the purpose, the PARP inhibitor may also be added to the culture medium during and/or after the contacting time or even solely after the contacting time.

In one aspect of this invention treatment of cultured cells, tissues or organs with PARP inhibitors may be used to increase the quality of transformation as measured by the copy number of the transgene and by variation in transgene expression (quality and quantity) in the transformed cells and in organisms obtained from the transformed cells.

In many conventional procedures for genetic transformation of eucaryotic cells, particularly plant cells, cultured cells, tissues or organs will be used as starting material and cells in such cultures will be contacted with foreign DNA comprising at least one gene of interest (i.e. the transgene) under conditions that will promote the uptake of the foreign DNA in the cells and the ultimate integration of the foreign DNA into the genome of the cells.

In one embodiment of the invention it is preferred that a PARP inhibitor is added to the culture medium for a period of at least 2-3 days, preferably at least about 3 days, prior to contacting the cells with the foreign DNA. The exact period in which the cultured cells are incubated in PARP inhibitor containing medium is believed not to be critical but should probably not exceed 4 weeks. It appears that 2-14 days, particularly 3-10 days, is an optimal period and best results were obtained with an incubation period of approximately 4 to 5 days prior to the contacting time. Generally it is believed that 4 days is a useful period for the PARP inhibitor to be added to the culture medium prior to the contacting time.

The concentration of the PARP inhibitor in the medium is also believed to have an effect on the inhibition of PARP, which varies depending on the nature of the cells (species, tissue explant, general culture conditions, etc.) However,

within certain concentration ranges, the effect is minimal, especially when the cultured cells are not incubated for longer than 14 days. The optimal concentration range of PARP inhibitor in the medium may vary depending on the species from which the tissue, cell or cell culture is derived, but 250 mg/l (about 2 mM) is believed to be a suitable concentration for many purposes (e.g. for use with material derived from wheat). However, when nicotinamide is used in combination with plant material derived from rice, the concentration of nicotinamide should preferably be between 500 mg/l (about 4 mM) and 1000 mg/l (approx. 8 mM). On the other hand, when nicotinamide is used in combination with plant material derived from corn, the concentration of nicotinamide should preferably be 100 mg/l. Likewise, a concentration of 100 mg/l is already effective for wheat-derived plant material, but higher concentrations may be used. The optimal concentration will depend on the nature of the specific PARP inhibitor used, particularly on its strength of inhibition (as measured by its Ki and/or by its percentage inhibition of PARP under standard conditions - Sims et al, supra). It was found for instance that the optimal concentration for nicotinamide is approximately 250 mg/l (i.e. about 2 mM) but it is believed that concentrations up to 1000 mg/l (approx. 8 mM) and as low as 150 mg/l (approx. 1.25 mM), even as low as 100mg/l can be used to good effect. Preferably the nicotinamide concentration should be between 200 and 300 mg/l, i.e. between approximately 1.5 mM and 2.5 mM. In similar conditions, the optimal concentration for more potent PARP inhibitors such as 3-methoxybenzamide is about 0.5 mM, but it is believed that concentrations up to 2 mM and as low as 0.1 mM can be used to good effect. Similar concentrations apply to other PARP inhibitors.

If incubation times of longer than 14 days are used it is believed that the PARP inhibitor concentration should be reduced below 2 mM (e.g. between 0.5 mM and 1.5 mM and particularly approximately 0.8 mM).

For other PARP inhibitors optimal concentrations can be easily established by experimentation in accordance with this invention.

During transformation it is not known whether the integration of the DNA into the genome of the cell occurs immediately after uptake of DNA by the cell. It may very well be that the foreign DNA exists as free DNA within the cell for a certain period after the contacting time. Therefore cultured cells may be further incubated in medium containing a PARP inhibitor during and, for a limited period of time after, contacting the cells with the foreign DNA. Again the length of the incubation period is not critical but is preferably 2-10 days, particularly approximately 4 days. It is preferred that the inhibitor concentration of the PARP inhibitor in the culture medium after the contacting time should be below 2 mM, between 0.8 and 1 mM. If the cells that are to be transformed are not obtained from a cell or tissue culture (e.g. when intact tissue of an organism is contacted directly with DNA, as for example described in WO 92/09696) the PARP inhibitor may still be applied to the target cells prior to the contacting time but the addition of the PARP inhibitor to the culture of the transformed cells during or after the contacting time is preferred.

As indicated above, PARP inhibitor treatment of cultured cells for at least 2-3 days increases the quality of transformation. Indeed the number of copies of the foreign DNA is expected to be generally lower and variation in expression profile (level - i.e. the quantity - of expression as well as spatial and time distribution - i.e. the quality - of expression in the transgenic organism) of the gene(s) of interest in the foreign DNA, due to position effects, is decreased. However, at least in this aspect of the invention, the efficiency of transformation can be decreased. The efficiency of transformation as used herein can be measured by the number of transformed cells (or transgenic organisms grown from individual transformed cells) that are recovered under standard experimental conditions (i.e. standardized or normalized with respect to amount

of cells contacted with foreign DNA, amount of delivered DNA, type and conditions of DNA delivery, general culture conditions etc.).

Therefore it is preferred that the invention is used with transformation procedures that already have a high efficiency, such as Agrobacterium mediated transformation of dicots and direct gene transfer in monocots, particularly cereals (e.g. electroporation or particle bombardment of compact embryogenic callus in cereals - see WO 92/09696). Indeed these transformation procedures are generally highly efficient but the quality of transformation is generally poor. Position effects are large and, especially with direct gene transfer, the copy number of the transgene is often exceptionally high making analysis and selection of optimal transformants, as well as further breeding with the transformants, difficult.

In another aspect of this invention treatment of cultured plant cells for a short period of time (i.e. 1 day to maximally 2 days) prior to, or after contacting the cells with DNA may be used to increase the efficiency of Agrobacterium mediated transformation of plant species, such as many monocots, particularly the major cereals such as wheat and corn, for which this method is generally inefficient. It is believed that treatment of cultured plant cells during the contacting time may result in a lower tranformation efficiency, and might therefore not be suitable for this aspect of this invention. Likewise, it is believed that for the purpose of this aspect of the invention, the optimal treatment with a PARP inhibitor is 1 day to maximally 2 days prior to the contacting time, or alternatively 1 to maximally 2 days after the contacting time. In this embodiment of the invention the contacting of the plant cells with the DNA should of course be understood as contacting the cells with an appropriate Agrobacterium strain harboring an artificial T-DNA containing the foreign DNA with the gene(s) of interest. In this embodiment of the invention the quality of transformation is expected not to be affected but this is generally deemed to be

of lesser importance since Agrobacterium mediated transformation, being a biological process, already results in a generally low copy number of the transgene in the transformed plant cells.

In accordance with this invention the addition of PARP inhibitors, such as niacinamide, to the culture medium of eucaryotic cells, can be used in combination with any known transformation procedure that requires cells, tissues or organs cultured in vitro as starting cells to be contacted with foreign DNA. The process of this invention is thus generally identical to existing conventional transformation methods except for the fact that at some times during the tissue culture of the cells, a PARP inhibitor is added to the culture medium.

The cell of a plant, particularly a plant capable of being infected with Agrobacterium such as most dicotyledonous plants (e.g. Brassica napus) and some monocotyledonous plants, can be transformed using a vector that is a disarmed Ti-plasmid containing the gene(s) of interest and carried by Agrobacterium. This transformation can be carried out using conventional procedures (EP 0,116,718; Deblaere et al, supra; Chang et al, 1994, The Plant Journal 5:551-558). Preferred Ti-plasmid vectors contain the foreign DNA between the border sequences, or at least located to the left of the right border sequence, of the T-DNA of the Ti-plasmid. Of course, other types of vectors can be used to transform the plant cell, using procedures such as direct gene transfer (as described, for example, in EP 0,233,247), pollen mediated transformation (as described, for example, in EP 0,270,356, PCT patent publication "WO" 85/01856, and US patent 4,684,611), plant RNA virus-mediated transformation (as described, for example, in EP 0,067,553 and US patent 4,407,956) and liposome-mediated transformation (as described, for example, in US patent 4,536,475). Cells of monocotyledonous plants such as the major cereals including corn, rice, wheat, barley, and rye, can be

transformed (e.g. by electroporation) using wounded or enzyme-degraded intact tissues capable of forming compact embryogenic callus (such as immature embryos in corn), or the embryogenic callus (such as type I callus in corn) obtained thereof, as described in WO 92/09696. In case the plant to be transformed is corn, other recently developed methods can also be used such as, for example, the method described for certain lines of corn by Fromm et al., 1990, Bio/Technology 8:833; Gordon-Kamm et al., 1990, Bio/Technology 2:603 and Gould et al., 1991, Plant Physiol. 95:426. In case the plant to be transformed is rice, recently developed methods can also be used such as, for example, the method described for certain lines of rice by Shimamoto et al., 1989, Nature 338:274; Datta et al., 1990, Bio/Technology 8:736; and Hayashimoto et al., 1990, Plant Physiol. 93:857; Hiei et al, 1994, The Plant Journal 6:271-282).

The transformed cell can be regenerated into a mature plant and the resulting transformed plant can be used in a conventional breeding scheme to produce more transformed plants with the same characteristics or to introduce the gene(s) of interest in other varieties of the same related plant species. Seeds obtained from the transformed plants contain the chimeric gene(s) of this invention as a stable genomic insert. Thus the gene(s) of interest when introduced into a particular line of a plant species can always be introduced into any other line by backcrossing.

In animals pluripotent embryonic or somatic stem cells can be used as target for transformation (Capecchi et al, 1989, TIG:5:70-76).

The transformed cells and organisms of any plant or animal species, produced by the process of this invention, contain the foreign DNA as a stable insert in their genome, particularly in regions of the genome that remain transcriptionally active in the untransformed cells that have been exposed to a PARP inhibitor in

accordance with this invention. As described above it is believed that in cells treated with a PARP inhibitor for at least 3 days, particularly for at least 4 days, only a limited number of genomic regions will remain transcriptionally active. In this regard the transformed cells, obtained with this process of the invention, will be characterized by having the foreign DNA integrated in a limited number of genomic regions. That the transformed cell or organism was obtained by this process of the invention can thus be easily ascertained by 1) culturing transformed cells or tissues under conditions that are similar as those in which the untransformed cells or tissues were grown or incubated prior to the integration of the foreign DNA in the genome (i.e. incubating in medium containing 250 mg/l niacinamide for 4-5 days prior to the contacting time), and 2) monitoring the expression of at least one transgene in the foreign DNA that is expected to be expressed under normal tissue culture conditions (i.e. a selectable marker gene under the control of a promoter that directs expression in tissue culture). Under the above conditions the transformed cells or tissues of this invention express the relevant transgene in the tissue culture at essentially the same levels whether or not a PARP inhibitor is present in the culture medium. It is thus expected that, for instance after 4-5 days of culturing of the transformed cells in medium containing the PARP inhibitor, mRNA levels are not signicantly decreased, i.e. do not become lower than 75%, preferably not become lower than 90%, when compared to the mRNA levels observed in cells cultured in medium not containing the inhibitor. Indeed, if the relevant transgene is integrated in other regions of the genome (i.e. in regions that are normally not transcriptionally active in cells treated with PARP inhibitor according to this embodiment of the invention), the expression of the relevant transgene is considerably reduced after incubation of the cells in medium containing the PARP inhibitor for at least 3 days, e.g. 4-5 days (i.e. mRNA levels will drop below 75%, particularly below 50%, more particularly below

30%) when compared to the mRNA levels observed in cells cultured in medium not containing the inhibitor).

The method of the present invention can in principle be used to transform eucaryotic cells with any foreign DNA. Generally the foreign DNA comprises at least one gene of interest comprising 1) a promoter region with a promoter capable of directing transcription of DNA into a RNA in cells of the eucaryotic, e.g. plant, species that is to be transformed and 2) a coding region coding for a RNA or protein. Most often the gene of interest will also comprise 3) a 3' untranslated region of a eucaryotic gene containing a polyadenylation signal.

The promoter can be selected to direct expression in selected tissues of the eucaryotic organism. Such a tissue-selective promoter is not expected to direct expression in other non-selected tissues. For instance promoters are known that direct expression selectively in stamen tissues of a plant and such promoters have been used to produce male sterile plants and other plants useful for producing hybrids (EP 344029; EP 412911; WO 9213956; WO 9213957; Mariani et al, 1990, Nature 347:737-741; Mariani et al, 1992, Nature 357:384-387).

It is believed that the method of the present invention is particularly useful to transform eucaryotic cells with at least one gene of interest comprising a tissue-selective promoter, such as a stamen selective promoter, especially if expression of the gene of interest in the organism, such as a plant, outside the selected tissues (where the tissue-selective promoter is active, i.e. directs expression) is undesired for example because the gene product (for instance a protein such as a ribonuclease, e.g. barnase) is capable of killing or disabling the cells in which they are produced. In such cases expression of the gene of interest in tissue culture, or in non-selected tissues of the organisms can negatively affect the quality as well as the apparent efficiency of transformation. When the method of this invention is used, the overall efficiency of

transformation may be reduced but the average quality of transformation is expected to be significantly improved because of lower copy number of the gene of interest in the genome of the transformed cells and because of reduced position effects i.e. the general integration of the gene of interest in the genomes at locations that minimally affect the transcriptional properties of the promoter of the transgene.

The foreign DNA used in the method of this invention generally also comprises a selectable marker gene the expression of which allows the selection of transformed cells (or organisms) from non-transformed cells (or organisms). Such selectable marker gene generally encodes a protein that confers to the cell resistance to an antibiotic or other chemical compound that is normally toxic for the cells. In plants the selectable marker gene may thus also encode a protein that confers resistance to a herbicide, such as a herbicide comprising a glutamine synthetase inhibitor (e.g. phosphinothricin) as an active ingredient. An example of such genes are genes encoding phosphinothricin acetyl transferase such as the sfr or sfrv genes (EP 242236; EP 242246; De Block et al, 1987 EMBO J 6:2513-2518).

The inventors also found that the initial reaction of cells, particularly cells contacted with PARP inhibitors, is a stress reaction which enhances free radical production by the cell. However, this stress only lasts for a limited period of time after which further contact with the PARP inhibitor causes a decrease in cell metabolism, particularly a decrease in electron flow in the mitochondrial electron transport chain. Therefore, the invention also relates to a new method to assess the agronomical fitness of a population of transformed plants to determine in which lines the plants have a foreign DNA integrated in their genomes in a way that agronomical performance is not or substantially not affected. The assay is based on comparative reaction of transgenic cells and corresponding untransformed controls to stress conditions.

The method comprises exposing the transgenic cells to stress conditions which induce the production of free radicals in the tissues or the cells, measuring the amount of free radicals produced in the transgenic cells with the amount of free radicals produced in control cells exposed to similar stress conditions. Preferably the cells of the transgenic organism to be assayed are exposed to stress conditions by being treated with a substance which induces increasing osmotic and/ or salt stress on the cells.

The properties of PARP inhibitors, such as niacinamide, to enhance free radical production in cells incubated with the inhibitor for not longer than 2 days, preferably not longer than 1 day, can be used to assay the (relative) fitness of a population of transgenic eucaryotic organisms, particularly plants.

The term fitness used herein is intended to designate the agronomical performance of a population of plants, as measured for instance by its yield (e.g. its seed yield) as compared to a given reference population. Agronomical performance is generally thought to be correlated with the general resistance of the plants to a range of stress conditions which are likely to be encountered in the field locations where the plants are normally grown. For any population of transformed plants (i.e. a transgenic line) the relevant reference population is a population of untransformed plants of the same variety.

It is known that in transformed plants and other organisms transgene expression may be qualitatively and quantitatively influenced by the genomic domain in which the transgene(s) are integrated, that undesired transgene expression may interfere with cell metabolism (e.g. when the transgene encodes a cytotoxic protein), that mutations may be induced in the transformed organism either by somaclonal variation or by insertional inactivation of

endogenous genes by the transgene(s), or that expression of endogenous genes may be dereguiated by sequences in the foreign DNA. As a consequence many transformed lines may not be agronomically useful.

The assay of this invention will for example allow to identify a line (i.e. a group of genetically similar plants) of transformed plants that have the transgene(s) integrated in regions that minimally affect the fitness of the plants, thus avoiding the extensive laboratory, greenhouse and/or field evaluations which are normally required to identify the transformants with the best agronomical properties.

The assay in accordance with this invention essentially comprises the incubation of cells or tissues of transformed plants of a particular transgenic line (e.g. callus, hypocotyl explants, shoots, leaf disks, whole leaves etc.) preferably with a PARP inhibitor (although for some plant species this is not necessary) under a range of conditions which induce the production of a different amount of free radicals in the tissues. An incubation time of approximately one day is normally sufficient to generate the desired amount of free radicals. Appropriate controls, i.e. untransformed tissues obtained from untransformed plants at the same developmental stage and grown in the same conditions as the transformed plant from which the transformed tissue was obtained, are subjected to the same treatment. Preferably the untransformed line is identical to the transgenic line except for the presence of the transgene(s).

For each plant line (control or transformant) it is preferred that a number of plants is assayed.

Useful conditions for the incubation of the untransformed and transformed tissues are those which induce increasing osmotic and salt stress in the incubated cells or tissues. For example a series of buffers with different salt

concentrations containing a PARP inhibitor can be made. A useful buffer series is a K-phosphate buffer containing 2% sucrose and 250 mg/l niacinamide in which the K-phosphate concentration is increased from anywhere between 10 to 80 mM (e.g. in steps of 5 mM, i.e. 10, 20, 25, 30, 35, 40, 45, 50, 55, 60 mM). The K-phosphate concentrations will induce mild but increasing salt and osmotic stress in plant cells. The niacinamide in the medium further enhances radical production and stress on the plant cells. The range of K-phosphate concentrations used will depend on the natural sensitivity of the plant species (or if desired the plant line) to the salt and osmotic stress. In sensitive plant species, which will not tolerate high salt stress, the maximum K-phosphate concentration may for instance be 50 mM, in less sensitive species this maximum K-phosphate concentration can be increased up to 70 or 80 mM or even higher. For each plant species the minimum and particularly the maximum salt (e.g. K-phosphate) concentration can be determined experimentally for an untransformed line - the only requirement is that at all concentrations used the plant tissue remains viable. Although the addition of a PARP inhibitor to the medium, such as niacinamide, is preferred it is not required for assaying plant species that are very sensitive to salt and/or osmotic stress.

After the one day incubation the capacity of the transformed and control tissues to reduce 2,3,5-triphenyltetrazolium chloride (TTC) is measured e.g. by the following procedure which is modified from Towill and Mazur (supra):

- incubate the tissues for 1 to 4 hours in K-phosphate buffer (pH 7.4) containing 10 mM TTC and 0.1 % Tween20. As a control similar plant material is incubated in the same buffer withour TTC.
- extraction of reduced TTC (e.g. freezing at -70°C followed by thawing at 40°C and shaking the plant material in ethanol for 45-60 minutes)

- spectrophotometric quantification of reduced TTC at 485 nm (optical density OD_{485}; for chlorophyll poor plant material) or 545 nm (OD_{545}; for chlorophyll rich plant material). The O.D. of the control extract is subtracted from the OD of the TTC-reacted extracts. In the above conditions 0.1 mM reduced TTC corresponds to an OD_{485} of 0.214 or OD_{545} of 1.025 (light path 1 cm).
- the reducing capacity of the transformed plant line is compared to that of the control line.

The amount of reduced TTC is determined by the intensity of the cytochromal and alternative respiratory pathways and the radical concentration in the tissues which, in turn are determined by the presence of mutations, the expression of genes affecting the metabolic activity of the plant cells, the developmental stage and the reaction of the tissue to external factors, such as stress factors.

The TTC reducing capacity (as for instance measured by the O.D. at 485 nm) for tissues incubated at high salt concentration (TTC-high) is expressed as the percentage of the TTC reducing capacity of the tissues incubated at a low salt concentration (TTC-low); in other words a TTC-ratio value is calculated as follows:

TTC-ratio = TTC/high.100/TTC.low.

The value of TTC-ratio is a measure of the fitness of a plant line as compared to a control line.

The determination of TTC-low and TTC-high will depend on the sensitivity of the plant species to the applied salt stress. Usually TTC-low will correspond to a salt concentration between 10 and 25 mM K-phosphate, e.g. at 20 mM while TTC-high will correspond to a salt concentration between 50 and 80 mM K-phosphate. The only requirement is that TTC-high should be significantly lower than TTC-low; preferably TTC-high should be lower than 50% of TTC-low, particularly lower than 30% of TTC-low. For instance for Brassica napus, TTC-

low and TTC-high can be typically obtained from tissues incubated at respectively 20mM and 60 mM K-phosphate buffer containing 250 mg/l niacinamide. TTC-high and TTC-low, for both the transformed and untransformed line, will usually be an average obtained from several measurements taken on a number of tissue explants from a number of plants of each line. For instance for each line of Brassica napus about 32 leaf discs (diameter 1 cm) from 8 different plants (i.e. about four leaf discs per plant) can be assayed to determine 32 TTC-high and 32 TTC-low values which are averaged to obtain the TTC-high and TTC-low values used for the calculation of TTC-ratio. Other examples of sample sizes which have been used are 35 shoots from Arabidopsis thaliana,or 150 hypocotyl explants derived from about 25 seedlings of Brassica napus.

Transformed lines with a value of TTC-ratio which does not deviate more than 20%, preferably not more than 10% of the TTC-ratio value of the control line are selected. These lines are likely to have the transgene(s) integrated in regions that minimally affect the fitness of the plants.

It is clear that additional information considering the fitness of the plant material studied can be obtained by comparing the TTC-reducing capacity of the plant material in absence of a PARP-inibitor with the TTC-reducing capacity of the plant material in the presence of a PARP-inibitor for each experimental point of the buffer series mentioned above.

While the TTC-reduction assay is especially suitable for the identification of transgenic plants, where transgenes are integrated in regions that minimally affect the fitness of the plants, this test can also be succesfully applied to discriminate mutant plants, cells or cell lines from the wild-types.

The TTC-reducing assay can further be used in a modified way to determine the quality and the fitness of plant material, for example plant material to be used in transformation experiments (i.e. whether particular plant material, e.g. explants, is suitable as starting material). To this end the TTC-reducing assay can be adapted for example in the following way:

1. A sample of the plant material to be tested for its suitability for transformation, is incubated for one day in plant culture medium or a buffer containing 2% sucrose and a K-phosphate concentration ranging between 10 and 80 mM, typically around 25 mM, to which a suitable amount of a PARP inhibitor, such as niacinamide has been added. For niacinamide, a preferred concentration to be used is 250 mg/L, although concentrations as low as 100 mg/L and as high as 1000 mg/L may be used. A comparable control sample of the same plant material is incubated under similar conditions without PARP inhibitor.
2. After one day of incubation the capacity of the plant material incubated with PARP inhibitor and the control plant material to reduce TTC is measured by the procedure described above.

The TTC reducing capacity (as for instance measured by the O.D. at 485 nm) for plant material incubated with PARP inhibitor (TTC-INH) is compared with the TTC reducing capacity of the control plant material incubated without PARP inhibitor (TTC-CON) and a ratio (E) is calculated as follows:

$$E = \text{TTC-INH} / \text{TTC-CON}$$

The value E is a measure of the quality and fitness of the plant material, for example explants to be transformed. It is believed that those tissues, wherein the E value is larger than or equals 1, are healthy tissues, which are particularly suitable as starting material for transformation.

The modified TTC-procedure thus allows to select those types of (cultured) plant material especially appropriate for use in a transformation procedure,

particularly the procedures of this invention that include the use of a PARP inhibitor.

As the quality of plant material will also be affected by the particular culture conditions used prior to transformation (especially cells, tissues or explants derived from plants recalcitrant to transformation)the assay of this invention is further useful to identify suitable culture conditions to obtain suitable starting plant material. Thus it has beeen found by the inventor that, when culturing plant material from corn, it is preferred to include proline, preferably at a concentration of about 8mM, simultaneously with the PARP inhibitor, in the culture medium.

As already mentioned, incubation of cells or tissues in the presence of a PARP inhibitor for longer than 1 to 2 days leads to a general reduction in cell metabolism, particularly a reduction in the electron flow in the mitochondrial electron transport chain (after the initial increase, characteristic of healthy cells or tissues, during the first day). The period of time required to reduce the metabolism to an optimal level (for the purpose of improving the qualitative aspect of transformation) is that period after which a decrease in TTC-reducing capacity between 20% and 50%, preferably between 30 % and 40%, particularly about 35%, is achieved for plant material incubated with a PARP inhibitor (e.g. niacinamide) when compared to control plant material incubated without the PARP inhibitor (i.e. the period after which the E value is between 0.5 and 0.8, preferably is between 0.6 and 0.7, particularly is about 0.65).

It is clear that the assays of this invention can be readily adapted by one skilled in the art of the field, for example to suit the needs of the particular cell type, tissue or explant or of the particular species from which the cells, tissues or explants are derived. Furthermore the assay can be adapted to assay a

peculiar aspect of fitness of cells, tissue, explant or organism. For instance, it is possible to apply a type of stress different from osmotic or salt stress, such as stress brought about by extreme temperatures, by sublethal treatment with chemicals (e.g. herbicides, heavy metals) or by irradiation with UV. Furthermore, other types of PARP inhibitors, as mentioned before may be used, within the indicated concentration ranges. Although it is believed that for the purpose of the assays defined here, TTC is the most suited substrate, other indicator molecules ,such as MTT (3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-2H-tetrazolium) can be used to measure the electron flow in the mitochondrial electron transport chain downstream of the "ubiquinone pool".

Unless otherwise indicated all experimental procedures for manipulating recombinant DNA were carried out by the standardized procedures described in Sambrook et al., 1989, "Molecular Cloning: a Laboratory Manual", Cold Spring Harbor Laboratory, and Ausubel et al, 1994, "Current Protocols in Molecular Biology", John Wiley & Sons.

The polymerase chain reactions ("PCR") were used to clone and/or amplify DNA fragments. PCR with overlap extension was used in order to construct chimeric genes (Horton et al, 1989, Gene 77:61-68; Ho et al, 1989, Gene 77:51-59).

All PCR reactions were performed under conventional conditions using the Vent™ polymerase (Cat. No. 254L - Biolabs New England, Beverley, MA 01915, U.S.A.) isolated from Thermococcus litoralis (Neuner et al., 1990, Arch.Microbiol. 153:205-207). Oligonucleotides were designed according to known rules as outlined for example by Kramer and Fritz (1968, Methods in Enzymology 154:350), and synthesized by the phosphoramidite method (Beaucage and Caruthers, 1981, Tetrahedron Letters 22:1859) on an applied Biosystems 380A DNA synthesizer (Applied Biosystems B.V., Maarssen,

Netherlands). In the examples MS medium means Murashige and Skoog medium (Murashige and Skoog, 1962, Physiol. Plant 15:473-479).

In the following examples, reference will be made to the following sequence listing and figures:

Sequence Listing

SEQ ID NO 1 : T-DNA of plasmid pTHW107
SEQ ID NO 2: plasmid pTS172
SEQ ID NO 3: PT72 promoter contained in plasmid pTS772
SEQ ID No 4 : plasmid pVE136
SEQ ID No 5 : T-DNA of plasmid pTHW142

Examples

Example 1 : Tissue culture of wheat embryogenic callus and Brassica napus hypocotyl explants in media containing a PARP inhibitor.

Wheat embryogenic callus was cultured on W2 medium (see Example 2). When niacinamide was added as PARP-inhibitor to the medium at a concentration of 250 mg/l (approx. 2 mM) it was observed that after 4 days the growth of the tissue was slowed down considerably (to approximately 30% of the normal rate after 4 weeks) but the tissue remained viable for extended periods of time (i.e. at least one month). If niacinamide was subsequently removed from the medium the tissue started to grow normally again. It was also observed that after 4-5 days of incubation of the plant tissue with niacinamide, the TTC-reducing capacity (Towill and Mazur, 1975, Can J.Bot. 53:1097-1102) of the tissue was substantially decreased probably indicating a reduction of the production of free radicals and decreased mitochondrial electron transport.

Similar observations were made when Brassica napus hypocotyl explants were cultured on A5 medium (see Example 3) containing 250 mg/l niacinamide. It was also observed that, in Brassica napus tissue cultured on medium containing niacinamide, no anthocyanin was produced; normally anthocyanin in tissue culture is produced in stress conditions. In addition it was observed that after 4-5 days of incubation of the plant tissue with niacinamide, the concentrations of hydroxyl free radical and dehydroascorbate in the explants were drastically decreased.

It was also observed that, after a 4 day incubation in niacinamide containing medium, the percentage of cultured cells that were in G2 phase of the cell cycle was considerably increased (up to 45 % of all cells in the culture).

The above observations are interpreted as indicating that treating cultured cells with a PARP inhibitor for about 4-5 days generally results in :

1) a significant reduction of the response of the cultured cells to stress as measured for instance by free radical and/or anthocyanin production , and

2) a reduction of the general metabolism of the cultured cells to a very basic level as indicated by the fact that the tissue growth was slowed down, and the TTC reducing capacity was decreased while the tissue remained viable.

It is inferred that under these conditions many genes in cells (e.g. cultured cells) that would normally be switched on in response to stress (such as during transformation conditions) will in fact no longer be induced. It is expected that in such cells which only display a very basic metabolism, mainly general "housekeeping genes", i.e. genes that are expressed in any cell irrespective of its differentiated state or metabolic or physiological condition, are expressed.

As it is believed that foreign DNA is preferably inserted in portions of the genome that are transcriptionally active it follows that treatment with PARP inhibitors will condition eucaryotic cells to incorporate any foreign DNA

preferentially in genomic regions which are transcribed in all cells and not in regions of the genome which would only be transcribed under certain conditions, i.e. stress conditions, or during differentiation. This means that the number of locations in which foreign DNA will be integrated, and the concomitant variation in expression profile of the transgene(s), will be reduced. It is further believed that this will enhance integration of foreign genes of interest in such locations which in turn will result in a more reliable and faithful expression of these genes which will be less affected by cell differentiation or cell physiological and biochemical changes due to for instance environmental conditions.

Example 2 : Transformation of wheat with a barnase gene under the control of a stamen-specific promoter using the particle bombardment

The Wheat Spring variety Pavon is grown in a greenhouse or conditioned room at 23-24°C during daytime and 18-20°C at night, with a photoperiod of 16 hours light and 8 hours dark. Developing seeds (white-greenish with white semi-liquid endosperm) were harvested, sterilized by incubation for 1 minute in 70% ethanol followed by 15 minute incubation in 1.3% NaOCl+ 0.1% Tween 20, and washed with sterile water. The sterilized seeds were either used directly or were stored for one day at 4-7°C.

Immature embryos of about 1 mm in size were isolated and were placed, with the scutellum upwards, on callus inducing medium W1 (MS medium supplemented with 3% sucrose, 40 mg/l adenine.SO_4, 0.5 mg/l thiamine.HCl, 0.5 g/l 2-[N-Morpholino] ethane sulfonic acid (Mes) pH 5.8, 0.5% agarose, 0.5 to 2.5 mg/l $CuSO_4.5H_2O$, 25 mg/l acetylsalicylic acid and 2 mg/l 2,4-dichlorophenoxyacetic acid (2,4-D)) and were incubated for 3 weeks at 27°C in the dark.

Embryogenic sections of the developing callus were isolated, placed on callus maintenance medium W2 (W1 medium but without acetylsalicylic acid and with

only 0.5 mg/l $CuSO_4.5H_20$ and 1 mg/l 2,4-D), and incubated for 3 weeks at 24-25°C in the light (approx. 20 mEinsteins/s/m^2 (with a photoperiod of 16 hours light and 8 hours dark).

About 2 weeks prior to bombardment the calli were cleaned up by removal of non-morphogenic (i.e. the nonembryogenic and nonmeristematic) parts and were subcultured on W2 medium.

For bombardment the calli were divided into small pieces with an average maximum diameter of about 2-3 mm. These pieces were placed at the center of a 9 cm Petridish containing W2 medium in a circle with a diameter of approx. 0.5 cm. When required niacinamide (250 mg/l) was added to the W2 medium and the tissue pieces were maintained under these conditions for 4 days after they were bombarded.

Bombardment was carried out using the Biolistic PDS-1000/He apparatus (Bio-Rad). Preparation of the microcarriers (0.4-1.2m) and the coating of the microcarriers with DNA was essentially carried out according to the manufacturer's instructions. The Petridishes containing the calli were placed at level 2 of the apparatus and the bombardment was done at 1550 psi.

For the transformation experiments the following plasmid DNA was used.

- plasmid pVE136, the sequence of which is given in SEQ ID No 4. This plasmid contains the following chimeric genes:
  - P35S-bar-3'nos
  - PCA55-barnase-3'nos

  in which P35S is the 35S promoter of the Cauliflower Mosaic virus, bar is a DNA encoding phosphinothricin acetyltransferase (EP 242236), 3'nos is the 3' untranslated end of the Agrobacterium T-DNA nopaline synthase gene, PCA55 is a stamen-specific promoter from corn gene CA55 (WO

9213957), and barnase is a DNA encoding barnase (Hartley, 1988, J.Mol.Biol.202:913-915)

- plasmid pTS172 the sequence of which is given in SEQ ID No 2. This plasmid contains the following chimeric genes:
  - P35S-bar-3'g7
  - PE1-barnase-3'nos

  in which in which P35S is the 35S promoter of the Cauliflower Mosaic virus, bar is a DNA encoding phosphinothricin acetyltransferase (EP 242236), 3'g7 is the 3' untranslated end of the Agrobacterium T-DNA gene 7, PE1 is a stamen-specific promoter from rice gene E1 (WO 9213956), barnase is a DNA encoding barnase (Hartley, 1988, J.Mol.Biol.202:913-915), and 3'nos is the 3' untranslated end of the Agrobacterium T-DNA nopaline synthase gene,
- plasmid pTS772 which is identical to pTS172 except that the region between nucleotides 2625-4313 of pTS172, containing PE1, is replaced by the sequence of SEQ ID No 3 containing the PT72 promoter. Thus, plasmid pTS772 contains the following chimeric genes:
  - P35S-bar-3'g7
  - PT72-barnase-3'nos

  in which PT72 is a stamen-specific promoter from rice gene T72 (WO 9213956)

The bombarded calli were transferred to selective medium W2 containing 2.5 mg/l phosphinothricin (PPT) and, if neccesary, 100 mg/l niacinamide. The calli that were placed on medium containing niacinamide were transferred after 4 days to niacinamide-free W2 medium containing 2.5 mg/l PPT. The cells were cultured at 24-25°C.

After two weeks the calli were subcultivated on W2 medium and after a further two weeks the growing parts of the calli were transferred to regeneration medium W4 (W1 medium but without acetylsalicylic acid and with only 0.5 mg/l

$CuSO_4.5H_20$ and 0.5 mg/l 2,4-D). Calli were subcultivated every two weeks at which time the nonmorphogenic parts of the calii were removed. When the calli started to form shoots they were transferred to W5 medium (W1 medium with half concentrated MS medium and only 0.5 mg/l $CuSO_4.5H_20$ and without acetylsalicylic acid and 2,4-D, but supplemented with 50 mg/l myo-inositol, 0.25 mg/l pyridoxine.HCl and 0.25 mg/l nicotinic acid) containing 2.5 mg/l PPT. For the rest of the procedure temperature was maintained at a maximum of 24°C. The calli were subcultivated every 3-4 weeks. Once the shoots started to elongate and small roots started to form, the whole calli (or if possible individual shoots) were transferred to 1 liter vessels with W6 medium (half-concentrated MS medium supplemented with 1.5% sucrose, 50 mg/l myo-inositol, 0.25 mg/l pyridoxine.HCl, 0.25 mg/l nicotinic acid, 0.5 mg/l thiamine.HCl, 0.7% agar (Difco) pH 5.8 and 0.5 mg/l $CuSO_4.5H_2O$) containing 2.5 mg/l PPT. Once the shoots and roots had grown out, individual shoots were separated from each other and transferred to 1 l vessels containing W6 medium with 2.5 mg/l PPT. Well developed shoots are tested for PPT resistance by means of the TLC assay (De Block et al, 1987, EMBO 6:2513-2518) or by direct assay of ammonium production in the tissue (see e.g. De Block et al, 1995, Planta 197: 619-626). Transformed shoots were finally transferred to the greenhouse into soil.

For analysis of the results the transformed plants could be subdivided according to the niacinamide treatment of the parent calli during tissue culture. Thus the following groups were distinguished:

Group	Niacinamide treatment
None	No treatment
Before 100	100 mg/l niacinamide for four days prior to bombardment
Before 250	250 mg/l niacinamide for four days prior to bombardment

Before/After 250 mg/l niacinamide for four days prior to bombardment plus 100 mg/l niacinamide for four days after bombardment

Results of the experiments are presented in Tables 1, 2 and 3. Plants could be obtained only from bombarded calli that were treated with niacinamide.
For the plants that were transformed with plasmid pTS172 it was demonstrated that the foreign DNA, comprising the chimeric PE1-barnase-3'nos and P35S-bar-3'g7, was stably incorporated in the wheat genome in 2 to 3 copies on the average. The fact that variation in expression profile (e.g. tissue-specificity) of the transgenes, especially the chimeric barnase genes, was decreased in transformed cells was evident from the fact that male-sterile plants that otherwise looked completely healthy could be obtained only from bombarded calli treated with niacinamide. It is believed that this is due to a more faithful expression characteristics (i.e. lack of expression) of the integrated stamen-selective barnase gene in these calli and shoots regenerated from these calli. In the control calli undesired expression of the barnase gene in tissue cultured cells might have prevented recovery of any transformed plants from these calli. It is expected that to obtain the same number of male-sterile wheat plants from control calli a much larger number of calli would have to be bombarded.

Results of wheat transformation experiments

Table 1 :

Plasmid pTS172				
Treatment	Nr of bombarded calli	Nr of PPT-resistant calli recovered	Nr of PPT resistant plants recovered	Nr of MS plants recovered
None	60	30	1[a)]	0
Before 250	125	30	3	3[b)]

a) This plant proved to be fertile and to be transformed only with the chimeric bar gene

b) The obtained plants looked healthy and tillered vigorously

Table 2:

Plasmid pTS772				
Treatment	Nr of bombarded calli	Nr of PPT-resistant calli recovered	Nr of PPT resistant plants recovered	Nr of MS plants recovered
None	250	22	0	0
Before 250	210	75	7	3[a)b)]
Before/ After	210	45	6	3[a]

a) The obtained plants looked healthy and tillered vigorously

b) Only six plants could be analyzed for MS phenotype since one of the plants died prematurely.

Table 3:

Plasmid pVE136			
Treatment	Nr of bombarded calli	Nr of PPT resistant plants recovered	Nr of MS plants recovered
None	200	1	0
Before 100	800	8[a)]	8

a) The obtained plants looked healthy and tillered vigorously

Example 3: Transformation of oilseed rape with a barnase gene under the control of a stamen-specific promoter using Agrobacterium mediated transformation.

Hypocotyl explants of Brassica napus were obtained , cultured and transformed essentially as described by De Block et al, 1989, Plant Physiol. 914:694-701 except for the following modifications:

- hypocotyl explants were precultured for 3 days on A2 medium (MS, 0.5 g/l Mes (pH 5.7), 1.2% glucose, 0.5% agarose, 1 mg/l 2,4-D, 0.25 mg/l naphthalene acetic acid (NAA), 1 mg/l 6-benzylaminopurine (BAP)), and then transferred to the A2 medium with or without niacinamide for another 4 days.
- infection medium A3 was MS, 0.5 g/l Mes (pH 5.7), 1.2% glucose, 0.1 mg/l NAA, 0.75 mg/l BAP, 0.01 mg/l giberellinic acid (GA3)
- selection medium A5 was 0.5 g/l Mes (pH 5.7), 1.2 % glucose, 40 mg/l adenine.SO_4, 0.5 g/l polyvinyl-polypyrrolidone (PVP), 0.5% agarose, 0.1 mg/l NAA, 0.75 mg/l BAP, 0.01 mg/l GA3, 250 mg/l carbenicillin, 250 mg/l triacillin, 5 mg/l $AgNO_3$.
- regeneration medium A6 was MS, 0.5 g/l Mes (pH 5.7), 2% sucrose, 40 mg/l adenine.SO_4, 0.5 g/l PVP, 0.5% agarose, 0.0025 mg/l BAP, 250 mg/l triacillin.
- healthy shoots were transferred to 1 liter vessels containing rooting medium which was either A8 or A9; A8 consists of 100-130 ml half concentrated MS, 1% sucrose (pH 5.0), 1 mg/l isobutyric acid (IBA), 100 mg/l triacillin added to 300 ml perlite (final pH 6.2); A9 consists of half concentrated MS, 1.5% sucrose (pH 5.8) solidified with agar (0.6%)

Hypocotyl explants (with or without niacinamide treatment) were infected with Agrobacterium tumefaciens strain C58C1Rif carrying T-DNA vector pTHW107 and a helper Ti-plasmid pMP90 (Koncz and Schell, 1986, Mol.Gen.Genet. 204:383-396)(or a derivative thereof).

Plasmid pTHW107 is a vector carrying a T-DNA comprising the following chimeric genes :

- PTA29-barnase-3'g7
- PSSU-bar-3'nos

in which PTA29 is the promoter of the TA29 gene of tobacco (EP 344029) and PSSU is the promoter of the gene of Arabidopsis thaliana encoding the small subunit of Rubisco. The complete sequence of the T-DNA of pTHW107 is presented in SEQ ID No 1.

Where required niacinamide (250 mg/l) was added to the media for the last 4 days prior to infection with Agrobacterium. Plants regenerated from transformed calli obtained on niacinamide cultured cells were observed to have a low copy number as well as to display less variation in the expression profile of the transgenes (results summarized in Table 4). Five plants regenerated from the calli obtained by transformation including niacinamide and five plants regenerated from the calli obtained by conventional transformation without niacinamide inclusion, were analyzed by Southern hybridization to determine the copy number of the transgenes, and were further analyzed for reproductive phenotype. In the non-treated group, a substantial number of regenerated plants proved not to have a transgene integrated in their nuclear DNA.

Table 4:

Treatment	Id. No.	Vegetative phenotype[a]	Reproductive phenotype[b]	Copy No. of the transgenes[c]	Phenotype of the F1-progeny[d]
no treatment	1	stressed	sterile	3	stressed/sterile
	2	stressed	sterile	4-6	ND
	3	stressed	sterile	3	stressed/sterile
	4	normal	sterile	1	normal/sterile
	5	stressed	(bud fall)	ND	ND
Before 250	1	normal	sterile	1	normal/sterile
	2	normal	sterile	3	normal/sterile
	3	normal	sterile	1	ND
	4	normal	sterile	3	ND
	5	normal	sterile	2	ND

a. Vegetatively stressed plants have a small size and flower early, leaves are oblong and dark green.

b. Reproductive phenotype regards male sterility; in flowers where the buds fell off prematurely this phenotype was not scored, except where some buds resulted in flowers.

c. Copy number of the transgenes was estimated by comparative Southern. ND: not determined.

d. F1-progeny was obtained by pollinating the transformed plants with pollen obtained from an untransformed N90-740 line. F1-Progeny resistant to phosphinotricin was scored for vegetative and reproductive phenotype.

Example 4: Agrobacterium-mediated transformation of oilseed rape using niacinamide in the culture medium.

Hypocotyl explants of Brassica napus were obtained as described in Example 3. Four groups of 200 hypocotyl explants each, were either not treated with niacinamide (indicated in table 4 as NONE), treated with 250 mg/l niacinamide for 1 day prior to infection with Agrobacterium (BEFORE), treated for 2 days during the infection with 250 mg/l niacinamide (DURING), or treated for 1 day after the Agrobacterium infection with 250 mg/l niacinamide (AFTER).

All hypocotyl explants were infected with Agrobacterium tumefaciens strain C58C1Rif carrying T-DNA vector pTHW142 and a helper Ti-plasmid pMP90 (Koncz and Shell, 1986 supra)(or a derivative thereof).

Plasmid pTHW142 is a vector carrying a T-DNA comprising the following chimeric genes:

- PSSU-bar-3'g7
- p35S-uidA-3'35S

In which uidA is a DNA encoding b-glucuronidase (Jefferson et al., 1986, Proc. Natl. Acad. Sci. USA 83, 8447-8451) and 3' 35S is the 3' untranslated end of the cauliflower mosaic virus 35S transcript.

The complete sequence of the T-DNA of pTHW142 is presented in SEQ ID No 5.

After the Agrobacterium infection, hypocotyl explants were transferred to selection medium A5, and if appropriate to A5 medium containing 250 mg/l niacinamide. The hypocotyl explants that were placed on medium containing niacinamide were transferred after 1 day to niacinamide-free selection medium A5. After 5 weeks on selective medium the number of transformed calli was scored. b-glucuronidase expression was verified in the obtained calli using established protocols (Jefferson et al.,1986). The results are summarized in

Table 5. Niacinamide treatment either before or after the Agrobacterium infection significantly increase the transformation efficiency.

Table 5:

Treatment	Transformation frequency[a]	Remarks[b]
NONE	16%	small, green calli
BEFORE	32%	large, green calli
DURING	16%	very small, light green calli
AFTER	29%	large, green calli developing shoots

a. Determined as the number of transformed calli (PPT-resitant and GUS-positive) developing per 100 hypocotyl explants

b. Size determination was as follows:

very small: callus diameter of approximately 1-2 mm

small: callus diameter of approximately 2-3 mm

large: callus diameter of approximately 5 mm

All publications cited in this application are hereby incorporated by reference.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:
(A) NAME: PLANT GENETIC SYSTEMS N.V.
(B) STREET: Plateaustraat 22
(C) CITY: Ghent
(E) COUNTRY: Belgium
(F) POSTAL CODE (ZIP): 9000
(G) TELEPHONE: 32 9 235 84 58
(H) TELEFAX: 32 9 223 19 23
(I) TELEX: 11.361 Pgsgen

(ii) TITLE OF INVENTION: Genetic Transformation using a PARP inhibitor

(iii) NUMBER OF SEQUENCES: 5

(iv) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.30 (EPO)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4946 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: T-DNA of plasmid pTHW107

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (1..25)
(D) OTHER INFORMATION:/label= RB
/note= "T-DNA right border"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (97..330)
(D) OTHER INFORMATION:/label= 3'g7
/note= "3' untranslated region containing the polyadenylation signal of gene 7 of Agrobacterium T-DNA "

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (331..882)
(D) OTHER INFORMATION:/label= bar
/note= "region coding for phosphinothricin acetyl transferase"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (883..2608)
(D) OTHER INFORMATION:/label= PSSU
/note= "promoter region of Rubisco small subunit gene of Arabidopsis thali..."

(ix) FEATURE:
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(B) LOCATION:complement (2658..3031)
(D) OTHER INFORMATION:/label= 3'nos
/note= "3' untranslated region containing the polyadenylation signal of the nopaline synthase gene of Agrobacterium T-DNA"

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(D) OTHER INFORMATION:/label= barnase
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(B) LOCATION:complement (3368..4876)
(D) OTHER INFORMATION:/label= PTA29
/note= "promoter region of TA29 gene of Nicotiana tabacum"

(ix) FEATURE:
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(D) OTHER INFORMATION:/label= LB
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(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

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GGGCGGTACC GGCAGGCTGA AGTCCAGCTG CCAGAAACCC ACGTCATGCC AGTTCCCGTG 420

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(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6548 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: plasmid pTS172

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (2019..2288)
(D) OTHER INFORMATION:/label= 3'nos
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(B) LOCATION:complement (2289..2624)
(D) OTHER INFORMATION:/label= barnase
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(A) NAME/KEY: -
(B) LOCATION:complement (2625..4313)
(D) OTHER INFORMATION:/label= PE1
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(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:4336..5710
(D) OTHER INFORMATION:/label= P35S
/note= "35S promoter region of Cauliflower mosaic virus"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:5711..6262
(D) OTHER INFORMATION:/label= bar
/note= "region coding for phosphinothricin acetyl transferase"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:6263..6496
(D) OTHER INFORMATION:/label= 3'g7
/note= "3' untranslated region containing the polyadenylation signal of gene 7 of Agrobacterium T-DNA"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

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CCGAGGGCTT GTGCTTCTGA TTTTGTAATG TAATTATCAG GTAGCTTATG ATATGTCTGA 2580

AGATAATCCG CAACCCCGTC AAACGTGTTG ATAACCGGTA CCATCGCGAC GGCTTGATGG 2640

ATCTCTTGCT GGACACCGGG ATGCTAGGAT GGGTTATCGT GGCCGGCGTG CGTGTGTGGC 2700

TTTTGTAGGC GCCGGCGACG GCGGGGGCAA TGTGGCAGGT GAGTCACGGT GCAAGCGTGC 2760

GCAAGTGACT GCAACAACCA AGGACGGTCA TGGCGAAAGC ACCTCACGCG TCCACCGTCT 2820

ACAGGATGTA GCAGTAGCAC GGTGAAAGAA GTGTTGTCCC GTCCATTAGG TGCATTCTCA 2880

CCGTTGGCCA GAACAGGACC GTTCAACAGT TAGGTTGAGT GTAGGACTTT TACGTGGTTA 2940

ATGTATGGCA AATAGTAGTA AATTTTGCCC CCATTGGTCT GGCTGAGATA GAACATATTC 3000

TGGAAAGCCT CTAGCATATC TTTTTTGACA GCTAAACTTT GCTTCTTGCC TTCTTGGTCT 3060

AGCAATGACG TTGCCCATGT CGTGGCAAAC ATCTGGTAAG GTAACTGTAT TCGTTTGTTC 3120

CCTTCAACGG CTCAATCCCC ACAGGCCAAG CTATCCTTTC CTTGGCAGTA TAGGCTCCTT 3180

GAGAGATTAT ACTACCATTT TTAAGTGCTT ATAAAGACGA TGCTCTCTAA CCAGATCGAT 3240

CAGAAACACA AAGTTTTAGC AGCGTAATAT CCCACACACA TACACACACG AAGCTATGCC 3300

TCCTCATTTT CCGAGAGATT CTGACAGTGA CCAGAATGTC AGAATGCCAT TTCATGGGCA 3360

CAAGTCGATC CACAAGCTTC TTGGTGGAGG TCAAGGTGTG CTATTATTAT TCGCTTTCTA 3420

GGAAATTATT CAGAATTAGT GCCTTTTATC ATAACTTCTC TCTGAGCCGA TGTGGTTTTG 3480

GATTTCATTG TTGGGAGCTA TGCAGTTGCG GATATTCTGC TGTGGAAGAA CAGGAACTTA 3540

TCTGCGGGGG TCCTTGCTGG GGCAACATTG ATATGGTTCC TGTTCGATGT AGTAGAATAC 3600

AATATAATTC CGCTCCTTTG CCAGATTGCC ATTCTTGCCA TGCTTGTGAT CTTCATTTGG 3660

TCAAATGCCG CACCACTCTT GGACAGGTAT TAGCTTTATT TCCTGTGGAG ATGGTAGAAA 3720

ACTCAGCTTA CAGAAATGGC ATTTCACGTA GTATAACGCA AGACATTAGG TACTAAAACT 3780

CAACTAACTG TTTCCGAATT TCAGGGCCCC TCCAAGGATC CCAGAAATCA TCATCTCTGA 3840

ACATGCCTTC AGAGAAATGG CATTGACCGT CCATTACAAA CTAACGTACA CTGTATCTGT 3900

TCTTTACGAC ATTGCATGTG GAAAGGATCT GAAGAGATTT CTCCTGGTAC ATAATAATCT 3960

ACTCCTTTGC TACGTTAATA AGAGATGTAA AAACATGCAA CAGTTCCAGT GCCAACATTG 4020

TCCAAGGATT GTGCAATTCT TTCTGGAGCG CTAAAATTGA CCAGATTAGA CGCATCAGAA 4080

TATTGAATTG CAGAGTTAGC CAATAATCCT CATAATGTTA ATGTGCTATT GTTGTTCACT 4140

ACTCAATATA GTTCTGGACT AACAATCAGA TTGTTTATGA TATTAAGGTG GTTGGATCTC 4200

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GGTATTCCAG ATAAATATTA AATTTTAATA AAACAATCAC ACAGAAGGAT CTGCGGCCGC 4320

TAGCCTAGGC CCGGGCCCAC AAAAATCTGA GCTTAACAGC ACAGTTGCTC CTCTCAGAGC 4380

AGAATCGGGT ATTCAACACC CTCATATCAA CTACTACGTT GTGTATAACG GTCCACATGC 4440

CGGTATATAC GATGACTGGG GTTGTACAAA GGCGGCAACA AACGGCGTTC CCGGAGTTGC 4500

ACACAAGAAA TTTGCCACTA TTACAGAGGC AAGAGCAGCA GCTGACGCGT ACACAACAAG 4560

TCAGCAAACA GACAGGTTGA ACTTCATCCC CAAAGGAGAA GCTCAACTCA AGCCCAAGAG 4620

CTTTGCTAAG GCCCTAACAA GCCCACCAAA GCAAAAAGCC CACTGGCTCA CGCTAGGAAC 4680

CAAAAGGCCC AGCAGTGATC CAGCCCCAAA AGAGATCTCC TTTGCCCCGG AGATTACAAT 4740

GGACGATTTC CTCTATCTTT ACGATCTAGG AAGGAAGTTC GAAGGTGAAG GTGACGACAC 4800

TATGTTCACC ACTGATAATG AGAAGGTTAG CCTCTTCAAT TTCAGAAAGA ATGCTGACCC 4860

ACAGATGGTT AGAGAGGCCT ACGCAGCAGG TCTCATCAAG ACGATCTACC CGAGTAACAA 4920

TCTCCAGGAG ATCAAATACC TTCCCAAGAA GGTTAAAGAT GCAGTCAAAA GATTCAGGAC 4980

TAATTGCATC AAGAACACAG AGAAAGACAT ATTTCTCAAG ATCAGAAGTA CTATTCCAGT 5040

ATGGACGATT CAAGGCTTGC TTCATAAACC AAGGCAAGTA ATAGAGATTG GAGTCTCTAA 5100

AAAGGTAGTT CCTACTGAAT CTAAGGCCAT GCATGGAGTC TAAGATTCAA ATCGAGGATC 5160

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ACAAGAAGAA AATCTTCGTC AACATGGTGG AGCACGACAC TCTGGTCTAC TCCAAAAATG 5280

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CGGGAAACCT CCTCGGATTC CATTGCCCAG CTATCTGTCA CTTCATCGAA AGGACAGTAG 5400

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TAAGGGATGA CGCACAATCC CACTATCCTT CGCAAGACCC TTCCTCTATA TAAGGAAGTT 5640

CATTTCATTT GGAGAGGACA CGCTGAAATC ACCAGTCTCT CTCTATAAAT CTATCTCTCT 5700

CTCTATAACC ATGGACCCAG AACGACGCCC GGCCGACATC CGCCGTGCCA CCGAGGCGGA 5760

CATGCCGGCG GTCTGCACCA TCGTCAACCA CTACATCGAG ACAAGCACGG TCAACTTCCG 5820

TACCGAGCCG CAGGAACCGC AGGAGTGGAC GGACGACCTC GTCCGTCTGC GGGAGCGCTA 5880

TCCCTGGCTC GTCGCCGAGG TGGACGGCGA GGTCGCCGGC ATCGCCTACG CGGGCCCCTG 5940

GAAGGCACGC AACGCCTACG ACTGGACGGC CGAGTCGACC GTGTACGTCT CCCCCCGCCA 6000

CCAGCGGACG GGACTGGGCT CCACGCTCTA CACCCACCTG CTGAAGTCCC TGGAGGCACA 6060

GGGCTTCAAG AGCGTGGTCG CTGTCATCGG GCTGCCCAAC GACCCGAGCG TGCGCATGCA 6120

CGAGGCGCTC GGATATGCCC CCCGCGGCAT GCTGCGGGCG GCCGGCTTCA AGCACGGGAA 6180

CTGGCATGAC GTGGGTTTCT GGCAGCTGGA CTTCAGCCTG CCGGTACCGC CCCGTCCGGT 6240

CCTGCCCGTC ACCGAGATCT GAGATCACGC GTTCTAGGAT CCCCCGATGA GCTAAGCTAG 6300

CTATATCATC AATTTATGTA TTACACATAA TATCGCACTC AGTCTTTCAT CTACGGCAAT 6360

GTACCAGCTG ATATAATCAG TTATTGAAAT ATTTCTGAAT TTAAACTTGC ATCAATAAAT 6420

TTATGTTTTT GCTTGGACTA TAATACCTGA CTTGTTATTT TATCAATAAA TATTTAAACT 6480

ATATTTCTTT CAAGATGGGA ATTAACATCT ACAAATTGCC TTTTCTTATC GACCATGTAC 6540

GTATCGCG 6548

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1601 base pairs
(B) TYPE: nucleic acid

(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: T72 promoter region

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (1..1601)
(D) OTHER INFORMATION:/label= PT72
/note= "promoter region of T72 gene of rice"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

CGCCGTGAGT GTCTTCTGCC GCCGAGGGGC TCTCGCTCGT CGTCGATGCC TGCACGGTGC 60

GTGCGTGTGT GTCGTGGTGG TGGTGGCGAT ACGCGACGCG AGCTCGATTT ATAGGAGGGG 120

ATCGAAGGAG GGGAGCGCGC GCGGCGAGGC CCGCGTTGCT CACCTACGCC GCGCGCATGC 180

GGCGGACGCG CGGTCGGCGC CCGCGCCGGC CGGGAGGACG AGGGCGCAAG CGTGTGAGCC 240

ACCGAACGCG CGCGCGCGCC GCGGCGCGAA CTCTCCATCG CGTCGCGGCG AGCCGAGAGC 300

CGACGAGAGC GTTTCGCGCG CGCGGTTGGG CCGGCGACAA GATGGGCCGT AGCCCTGGGC 360

CTCGTGCCAT CTTTTTTTTT CTTTTTTGCC TTTTTTGGCC TGGCAATTTC TTTTTGTTTT 420

TAGTCTTTTT GTGGTGATAA TGTGTCGTCT TCCGGTGAAC TAATTTACTC GTTGATCTTT 480

TTGTGTCCCT TCGAATATTC GCAGTGGTAG AAGATGACTA CTACTACCAG TAGTTGATCT 540

CGAATGGCAA CTTTTGTGCA GAACTTATTC CACGGCTATG TCAGCTTCCA CTGTGACTAA 600

AAAAACTACG GCCATCTTTT GGACTTGTTC TATCTTGGAA CTGAACAAAA AGGACGATCC 660

TGATGTACAC ACGGCATAGT TTCCAGCACT GGATGCCAAG TTGCCAACTG TTACCACGAT 720

AATGGAACGA CGAGATGAGA TATTATACAA GTCCAATGGA TCAAGATCCT GTGCAGTTGT 780

TATTGTAACT GTAACTTAAG CCGTTAACAT GTACATCACA TTTCCTACTC TATCAATGTC 840

TTGTGCGGGT TGTTTCAAAA AAACATGTAC ATCACATGAT CTAGAACGGA AGGCCAGGAT 900

ATGAAGTGGT ACTGCAGCAA AAACACTGTA GCAGAGATGT ACTATTATGC ATGTACTGTA 960

GCAGTCATCT AGAGCCGTTG GATCTGAAAA CGAATGGACA TGATTGTGTG CAGTTGCTAT 1020

TGTGCAGTTA CAATAGCAAC TGCATTTGAT CTTAATCCAA GTCCAATACA TGCAGAACAG 1080

TAGCTACGAG CTGGAAAGGA TGCAAATCTG GGTGACACTG ACAGCAACCG TGGAAGAACA 1140

ACAGCAGCAA AGTCCCAGAG GGATGGCAAT TTGAAGGAAT TTAAATACTC TAATATTACT 1200

CCACCCGTTA AAAAAAACAA CTTGCTACGC ATAATATATG TTCGGATTTA TAGCGAGAAG 1260

TTAATTTTTC ATGAGAAGAA GAATATATAT GTAATATGTA CTAGGAGAGT ACTCGCTTCA 1320

TAAATATAAA TATTCATAAG TTGTCCAGTG AAGATAGCTT TAGAAAAAAC TAGTTATTTT 1380

ATTTGTCAAA TTTTAAATTT TGAAGTAGTT AGATTATCTT TCTAGTAGTT CTGATTGGTT 1440

GAAAATGTTT AGATTTTCAT GTGTTAAGAG TTCCGTATCC TAAAAATAGT AATATAATTT 1500

TAAATCATAT ATATATATAT ATATATATAT ATATATATAT ATATATATAT ATATATATAT 1560

TGTTGAACGG TTTGTGCTCT GGTTGCTATC CTGTTCTGTG G 1601

(2) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 6291 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: circular

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: plasmid pVE136

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (425..687)
(D) OTHER INFORMATION:/label= 3'nos
/note= "3'untranslated region containing the polyadenylation signal of the nopaline synthase gene of Agrobacterium T-DNA"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (803..1138)
(D) OTHER INFORMATION:/label= barnase
/note= "region coding for barnase"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (1138..2317)
(D) OTHER INFORMATION:/label= PCa55

/note= "stamen-specific promoter from corn gene CA55"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:2355..3187
(D) OTHER INFORMATION:/label= P35S
/note= "35S promoter region of Cauliflower mosaic virus"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:3188..3739
(D) OTHER INFORMATION:/label= bar
/note= "region coding for phosphinotricin acetyl transferase"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:3757..4017
(D) OTHER INFORMATION:/label= 3'nos
/note= "3' untranslated region containing the polyadenylation
signal of the nopaline synthase gene of Agrobacterium T-DNA"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:699..702
(D) OTHER INFORMATION:/note= "region with unknown
sequence (may contain up to 15 nucleotides)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

TCGCGCGTTT CGGTGATGAC GGTGAAAACC TCTGACACAT GCAGCTCCCG GAGACGGTCA 60

CAGCTTGTCT GTAAGCGGAT GCCGGGAGCA GACAAGCCCG TCAGGGCGCG TCAGCGGGTG 120

TTGGCGGGTG TCGGGGCTGG CTTAACTATG CGGCATCAGA GCAGATTGTA CTGAGAGTGC 180

ACCATATGCG GTGTGAAATA CCGCACAGAT GCGTAAGGAG AAAATACCGC ATCAGGCGCC 240

ATTCGCCATT CAGGCTGCGC AACTGTTGGG AAGGGCGATC GGTGCGGGCC TCTTCGCTAT 300

TACGCCAGCT GGCGAAAGGG GGATGTGCTG CAAGGCGATT AAGTTGGGTA ACGCCAGGGT 360

TTTCCCAGTC ACGACGTTGT AAAACGACGG CCAGTGAATT CGAGCTCGGT ACCCGGGGAT 420

CTTCCCGATC TAGTAACATA GATGACACCG CGCGCGATAA TTTATCCTAG TTTGCGCGCT 480

ATATTTTGTT TTCTATCGCG TATTAAATGT ATAATTGCGG GACTCTAATC ATAAAAACCC 540

ATCTCATAAA TAACGTCATG CATTACATGT TAATTATTAC ATGCTTAACG TAATTCAACA 600

GAAATTATAT GATAATCATC GCAAGACCGG CAACAGGATT CAATCTTAAG AAACTTTATT 660

GCCAAATGTT TGAACGATCT GCTTCGGATC CTCTAGAGNN NNCCGGAAAG TGAAATTGAC 720

CGATCAGAGT TTGAAGAAAA ATTTATTACA CACTTTATGT AAAGCTGAAA AAAACGGCCT 780

CCGCAGGAAG CCGTTTTTTT CGTTATCTGA TTTTTGTAAA GGTCTGATAA TGGTCCGTTG 840

TTTTGTAAAT CAGCCAGTCG CTTGAGTAAA GAATCCGGTC TGAATTTCTG AAGCCTGATG 900

TATAGTTAAT ATCCGCTTCA CGCCATGTTC GTCCGCTTTT GCCCGGGAGT TTGCCTTCCC 960

TGTTTGAGAA GATGTCTCCG CCGATGCTTT TCCCCGGAGC GACGTCTGCA AGGTTCCCTT 1020

TTGATGCCAC CCAGCCGAGG GCTTGTGCTT CTGATTTTGT AATGTAATTA TCAGGTAGCT 1080

TATGATATGT CTGAAGATAA TCCGCAACCC CGTCAAACGT GTTGATAACC GGTACCATGG 1140

CTGCAGCTAG TTAGCTCGAT GTATCTTCTG TATATGCAGT GCAGCTTCTG CGTTTTGGCT 1200

GCTTTGAGCT GTGAAATCTC GCTTTCCAGT CCCTGCGTGT TTTATAGTGC TGTACGTTCG 1260

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GGTTTCATCC AGCCAGGAGA CCCGAATCGA ATTGAAATCA CAAATCTGAG GTACAGTATT 1440

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AGTGGAGTTT CAGCATTGAC GACGAAAACC TTCGAACGGT ATAAAAAAGA AGCCGCAATT 1680

AAACGAAGAT TTGCCAAAAA GATGCATCAA CCAAGGGAAG ACGTGCATAC ATGTTTGATG 1740

AAAACTCGTA AAAACTGAAG TACGATTCCC CATTCCCCTC CTTTTCTCGT TTCTTTTAAC 1800

TGAAGCAAAG AATTTGTATG TATTCCCTCC ATTCCATATT CTAGGAGGTT TTGGCTTTTC 1860

ATACCCTCCT CCATTTCAAA TTATTTGTCA TACATTGAAG ATATACACCA TTCTAATTTA 1920

TACTAAATTA CAGCTTTTAG ATACATATAT TTTATTATAC ACTTAGATAC GTATTATATA 1980

AAACACCTAA TTTAAAATAA AAAATTATAT AAAAAGTGTA TCTAAAAAAT CAAAATACGA 2040

CATAATTTGA AACGGAGGGG TACTACTTAT GCAAACCAAT CGTGGTAACC CTAAACCCTA 2100

TATGAATGAG GCCATGATTG TAATGCACCG TCTGATTAAC CAAGATATCA ATGGTCAAAG 2160

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CAGGCATGCA AGCTCCTACG CAGCAGGTCT CATCAAGACG ATCTACCCGA GTAACAATCT 2400

CCAGGAGATC AAATACCTTC CCAAGAAGGT TAAAGATGCA GTCAAAAGAT TCAGGACTAA 2460

TTGCATCAAG AACACAGAGA AAGACATATT TCTCAAGATC AGAAGTACTA TTCCAGTATG 2520

GACGATTCAA GGCTTGCTTC ATAAACCAAG GCAAGTAATA GAGATTGGAG TCTCTAAAAA 2580

GGTAGTTCCT ACTGAATCTA AGGCCATGCA TGGAGTCTAA GATTCAAATC GAGGATCTAA 2640

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AAGATACAGT CTCAGAAGAC CAAAGGGCTA TTGAGACTTT TCAACAAAGG ATAATTTCGG 2820

GAAACCTCCT CGGATTCCAT TGCCCAGCTA TCTGTCACTT CATCGAAAGG ACAGTAGAAA 2880

AGGAAGGTGG CTCCTACAAA TGCCATCATT GCGATAAAGG AAAGGCTATC ATTCAAGATG 2940

CCTCTGCCGA CAGTGGTCCC AAAGATGGAC CCCCACCCAC GAGGAGCATC GTGGAAAAAG 3000

AAGACGTTCC AACCACGTCT TCAAAGCAAG TGGATTGATG TGACATCTCC ACTGACGTAA 3060

GGGATGACGC ACAATCCCAC TATCCTTCGC AAGACCCTTC CTCTATATAA GGAAGTTCAT 3120

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TATAACCATG GACCCAGAAC GACGCCCGGC CGACATCCGC CGTGCCACCG AGGCGGACAT 3240

GCCGGCGGTC TGCACCATCG TCAACCACTA CATCGAGACA AGCACGGTCA ACTTCCGTAC 3300

CGAGCCGCAG GAACCGCAGG AGTGGACGGA CGACCTCGTC CGTCTGCGGG AGCGCTATCC 3360

CTGGCTCGTC GCCGAGGTGG ACGGCGAGGT CGCCGGCATC GCCTACGCGG GCCCCTGGAA 3420

GGCACGCAAC GCCTACGACT GGACGGCCGA GTCGACCGTG TACGTCTCCC CCCGCCACCA 3480

GCGGACGGGA CTGGGCTCCA CGCTCTACAC CCACCTGCTG AAGTCCCTGG AGGCACAGGG 3540

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GGCGCTCGGA TATGCCCCCC GCGGCATGCT GCGGGCGGCC GGCTTCAAGC ACGGGAACTG 3660

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GCCCGTCACC GAGATCTGAT CTCACGCGTC TAGGATCCGA AGCAGATCGT TCAAACATTT 3780

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TTCTGTTGAA TTACGTTAAG CATGTAATAA TTAACATGTA ATGCATGACG TTATTTATGA 3900

GATGGGTTTT TATGATTAGA GTCCCGCAAT TATACATTTA ATACGCGATA GAAAACAAAA 3960

TATAGCGCGC AAACTAGGAT AAATTATCGC GCGCGGTGTC ATCTATGTTA CTAGATCGGG 4020

AAGATCCTCT AGAGTCGACC TGCAGGCATG CAAGCTTGGC GTAATCATGG TCATAGCTGT 4080

TTCCTGTGTG AAATTGTTAT CCGCTCACAA TTCCACACAA CATACGAGCC GGAAGCATAA 4140

AGTGTAAAGC CTGGGGTGCC TAATGAGTGA GCTAACTCAC ATTAATTGCG TTGCGCTCAC 4200

TGCCCGCTTT CCAGTCGGGA AACCTGTCGT GCCAGCTGCA TTAATGAATC GGCCAACGCG 4260

CGGGGAGAGG CGGTTTGCGT ATTGGGCGCT CTTCCGCTTC CTCGCTCACT GACTCGCTGC 4320

GCTCGGTCGT TCGGCTGCGG CGAGCGGTAT CAGCTCACTC AAAGGCGGTA ATACGGTTAT 4380

CCACAGAATC AGGGGATAAC GCAGGAAAGA ACATGTGAGC AAAAGGCCAG CAAAAGGCCA 4440

GGAACCGTAA AAAGGCCGCG TTGCTGGCGT TTTTCCATAG GCTCCGCCCC CCTGACGAGC 4500

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GCAGAAAAAA AGGATCTCAA GAAGATCCTT TGATCTTTTC TACGGGGTCT GACGCTCAGT 5100

GGAACGAAAA CTCACGTTAA GGGATTTTGG TCATGAGATT ATCAAAAAGG ATCTTCACCT 5160

AGATCCTTTT AAATTAAAAA TGAAGTTTTA AATCAATCTA AAGTATATAT GAGTAAACTT 5220

GGTCTGACAG TTACCAATGC TTAATCAGTG AGGCACCTAT CTCAGCGATC TGTCTATTTC 5280

GTTCATCCAT AGTTGCCTGA CTCCCCGTCG TGTAGATAAC TACGATACGG GAGGGCTTAC 5340

CATCTGGCCC CAGTGCTGCA ATGATACCGC GAGACCCACG CTCACCGGCT CCAGATTTAT 5400

CAGCAATAAA CCAGCCAGCC GGAAGGGCCG AGCGCAGAAG TGGTCCTGCA ACTTTATCCG 5460

CCTCCATCCA GTCTATTAAT TGTTGCCGGG AAGCTAGAGT AAGTAGTTCG CCAGTTAATA 5520

GTTTGCGCAA CGTTGTTGCC ATTGCTACAG GCATCGTGGT GTCACGCTCG TCGTTTGGTA 5580

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TGTTATCACT CATGGTTATG GCAGCACTGC ATAATTCTCT TACTGTCATG CCATCCGTAA 5760

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GACCGAGTTG CTCTTGCCCG GCGTCAATAC GGGATAATAC CGCGCCACAT AGCAGAACTT 5880

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TGTTGAGATC CAGTTCGATG TAACCCACTC GTGCACCCAA CTGATCTTCA GCATCTTTTA 6000

CTTTCACCAG CGTTTCTGGG TGAGCAAAAA CAGGAAGGCA AAATGCCGCA AAAAAGGGAA 6060

TAAGGGCGAC ACGGAAATGT TGAATACTCA TACTCTTCCT TTTTCAATAT TATTGAAGCA 6120

TTTATCAGGG TTATTGTCTC ATGAGCGGAT ACATATTTGA ATGTATTTAG AAAAATAAAC 6180

AAATAGGGGT TCCGCGCACA TTTCCCCGAA AAGTGCCACC TGACGTCTAA GAAACCATTA 6240

TTATCATGAC ATTAACCTAT AAAAATAGGC GTATCACGAG GCCCTTTCGT C 6291

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 5560 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: double
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: DNA (genomic)

(iii) HYPOTHETICAL: NO

(iv) ANTI-SENSE: NO

(vi) ORIGINAL SOURCE:
(A) ORGANISM: T-DNA of plasmid pTHW142

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:1..25
(D) OTHER INFORMATION:/label= RB
/note= "right border sequence of octopine TL-DNA from pTiB6S3"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (84..296)
(D) OTHER INFORMATION:/label= 3'g7
/note= "3' untranslated region containing the polyadenylation signal of gene 7 of Agrobacterium T-DNA"

(ix) FEATURE:

(A) NAME/KEY: -
(B) LOCATION:complement (318..869)
(D) OTHER INFORMATION:/label= bar
/note= "region coding for posphinotricin acetyl transferase"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (830..2760)
(D) OTHER INFORMATION:/label= PSSU
/note= "promoter region of Rubisco small subunit gene of Arabidopsis thali..."

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (2765..3058)
(D) OTHER INFORMATION:/label= 3'35S
/note= "3' untranslated region of the CaMV 35S transcript containing polyadenylation signals"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (3059..5056)
(D) OTHER INFORMATION:/label= uidA
/note= "region coding for beta-glucoronidase"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (4483..4671)
(D) OTHER INFORMATION:/label= IV2
/note= "region corresponding to the second intron of the ST-LS1 gene"

(ix) FEATURE:
(A) NAME/KEY: -
(B) LOCATION:complement (5067..5502)
(D) OTHER INFORMATION:/label= P35S
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CLAIMS

1. A process for producing transgenic eucaryote cells which comprises:
contacting a culture of untransformed cells with an inhibitor of poly-(ADP-ribose) polymerase, prior to transformation, for a period of time sufficient to reduce the response of the cultured cells to stress and to reduce the metabolism of said cultured cells, particularly to reduce the electron flow in the mitochondrial electron transport chain; contacting said untransformed cells with foreign DNA comprising at least one gene of interest under conditions in which said foreign DNA is taken up by said untransformed cells and said gene of interest is stably integrated in the nuclear genome of said untransformed cells to produce said transgenic cells; and
optionally recovering said transgenic cells from said culture.

2. The proces of claim 1, wherein said eucaryotic cells are plant cells.

3. The process of claim 1 or 2, wherein said inhibitor is niacinamide, preferably at a concentration of about 150 mg/l to 1000 mg/l, more preferably at a concentration of about 200 mg/l to 500 mg/l, particularly at a concentration of about 250 mg/l.

4.The process of any one of claims 1 to 3, wherein said untransformed cells are cultured in a medium containing said inhibitor for a period of time of approximately 2 to 28 days, preferably approximately 3 to 14 days, particularly approximately 4 days prior to the contacting with said foreign DNA.

5. The process of any one of claims 1 to 4, wherein said cells contacted with said foreign DNA are further cultured in a medium containing said inhibitor

for a period of time of approximately 1 to 14 days, preferably 2 to 4 days after contacting with said foreign DNA.

6. A process for increasing the frequency of obtaining transgenic plant cells which comprises:

contacting untransformed plant cells with foreign DNA comprising at least one gene of interest under conditions in which said foreign DNA is taken up by said untransformed cells and said gene of interest is stably integrated in the nuclear genome of said untransformed cells to produce said transgenic cells

contacting cells with an inhibitor of poly-(ADP-ribose); and

further culturing said cells in a medium containing said inhibitor for a period of time of approximately 1 to 14 days, preferably 1 to 4 days, particularly 1 day after contacting with said foreign DNA.

7. The proces of any of claims 1 to 6, wherein said gene of interest comprises a promoter that directs expression selectively in certain cells or tissues of an eucaryotic organism.

8. The process of any one of claims 2 to 7, wherein said gene of interest comprises a promoter that directs expression selectively in stamen cells, particularly anther cells of a plant.

9. The process of claim 7 or 8, wherein said gene of interest encodes a protein that, when produced in a cell of an eucaryotic organism, kills or disables said cell.

10. The process of claim 9, wherein said gene of interest encodes a ribonuclease, particularly barnase.

11. The process of any one of claims 1 to 10, wherein a transgenic organism having said foreign DNA with said at least one gene of interest stably integrated in its genome is obtained from said transformed eucaryotic cell.

12. The proces of claim 11, wherein said organism is a plant which is obtained by regeneration from a transformed plant cell.

13. The transgenic organism obtained by the process of claim 11 or 12.

14. A plant having foreign DNA integrated in the nuclear DNA of its cells only in the regions of said nuclear DNA that are transcriptionally active in said cells of said plant when said cells are treated with an effective amount of a PARP inhibitor for a period of time sufficient to reduce cell metabolism to a state where gene expression is essentially limited to genes expressed irrespective of the differentiated or physiological condition of the cell.

15. The plant according to claim 14, wherein said integration of the foreign DNA in said transcriptionally active region is verified by measuring the level of expressed mRNA corresponding to this foreign DNA when said cells are incubated in a medium containing a PARP-inhibitor.

16. The plant according to claim 14, wherein said transcriptionally active regions of the genome of said plant include regions which are minimally affected by cell differentiation or cell physiological and biochemical changes caused by external factors such as environmantal conditions, especially stress conditions.

17. The plant or plant cell according to any one of the preceeding claims, wherein said plant or plant cell is a monocotyledonous plant or plant cell.

18. The plant or plant cell according to claim 17, wherein said plant or plant cell is a cereal plant or plant cell.

19. The plant of plant cell acccording to claim 18, wherein said plant or plant cell is wheat or a wheat cell.

20. The plant according to any one of the preceeding claims, wherein said foreign DNA comprises a DNA sequence expressed selectively in specific tissues of said plant.

21. The plant of claim 20, wherein said foreign DNA comprises a DNA sequence encoding a cytotoxic molecule.

22. The plant of claim 21, wherein said foreign DNA comprises a DNA sequence encoding barnase.

23. A eucaryotic cell having foreign DNA integrated in its nuclear DNA only in the regions of said nuclear DNA that are transcriptionally active in said cell when said cell is treated with an effective amount of a PARP inhibitor for a period of time sufficient to reduce cell metabolism to a state where gene expression is essentially limited to genes expressed irrespective of the differentiated or physiological condition of the cell.

BIRCH, S EWART, KOLASCH & BIRC. LLP

COMBINED DECLARATION AND POWER OF ATTORNEY FOR PATENT AND DESIGN APPLICATIONS

ATTORNEY DOCKET NO.
2121-127P

PLEASE NOTE: YOU MUST COMPLETE THE FOLLOWING:

As a below named inventor, I hereby declare that: my residence, post office address and citizenship are as stated next to my name; that I verily believe that I am the original, first and sole inventor (if only one inventor is named below) or an original, first and joint inventor (if plural inventors are named below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:*

Insert Title ➡ GENETIC TRANSFORMATION USING A PARP INHIBITOR

Check Box If Appropriate - For Use Without Specification Attached ➡

the specification of which is attached hereto unless the following box is checked:

☐ was filed on _____ as United States Application Number _____ or PCT International Application Number PCT/EP96/03366 filed July 31, 1996 and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above identified specification, including the claims, as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56.

I do not know and do not believe the same was ever known or used in the United States of America before my or our invention thereof, or patented or described in any printed publication in any country before my or our invention thereof, or more than one year prior to this application, that the same was not in public use or on sale in the United States of America more than one year prior to this application, that the invention has not been patented or made the subject of an inventor's certificate issued before the date of this application in any country foreign to the United States of America on an application filed by me or my legal representatives or assigns more than twelve months (six months for designs) prior to this application, and that no application for patent or inventor's certificate on this invention has been filed in any country foreign to the United States of America prior to this application by me or my legal representatives or assigns, except as follows.

I hereby claim foreign priority benefits under Title 35, United States Code, §119 (a)-(d) of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

Insert Priority Information (if appropriate) ➡

Prior Foreign Application(s)			Priority Claimed	
95401844.6 (Number)	United Kingdom (Country)	08/04/95 (Month/Day/Year Filed)	☒ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Month/Day/Year Filed)	☐ Yes	☐ No

I hereby claim the benefit under Title 35, United States Code, § 119(e) of any United States provisional application(s) listed below.

(Application Number) (Filing Date)

(Application Number) (Filing Date)

All Foreign Applications, if any, for any Patent or Inventor's Certificate Filed More Than 12 Months (6 Months for Designs) Prior To The Filing Date of This Application:

Country	Application No.	Date of Filing (Month/Day/Year)

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose information which is material to patentability as defined in Title 37, Code of Federal Regulations, §1.56 which became available between the filing date of the prior application and the national or PCT international filing date of this application:

(Application Number)	(Filing Date)	(Status — patented, pending, abandoned)

*NOTE: Must be completed.

I hereby appoint the following attorneys to prosecute this application and/or an international application based on this application and to transact all business in the Patent and Trademark Office connected therewith and in connection with the resulting patent based on instructions received from the entity who first sent the application papers to the attorneys identified below, unless the inventor(s) or assignee provides said attorneys with a written notice to the contrary:

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GERALD M. MURPHY, JR. (Reg. No. 28,977)
TERRY L. CLARK (Reg. No. 32,644)
ANDREW D. MEIKLE (Reg. No. 32,868)
ANDREW F. REISH (Reg. No. 33,443)

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Facsimile: (703) 205-8050

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Full Name of First or Sole Inventor:
Insert Name of Inventor
Insert Date This Document Is Signed
Insert Residence
Insert Citizenship
Insert Post Office Address

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Residence (City, State & Country)	CITIZENSHIP

POST OFFICE ADDRESS (Complete Street Address including City, State & Country)

Full Name of Third Inventor, if any:

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GIVEN NAME	FAMILY NAME	INVENTOR'S SIGNATURE	DATE*

Residence (City, State & Country)	CITIZENSHIP

POST OFFICE ADDRESS (Complete Street Address including City, State & Country)

Full Name of Fourth Inventor, if any:

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GIVEN NAME	FAMILY NAME	INVENTOR'S SIGNATURE	DATE*

Residence (City, State & Country)	CITIZENSHIP

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Full Name of Fifth Inventor, if any:

see above

GIVEN NAME	FAMILY NAME	INVENTOR'S SIGNATURE	DATE*

Residence (City, State & Country)	CITIZENSHIP

POST OFFICE ADDRESS (Complete Street Address including City, State & Country)

***Note:** Must be completed — date this document is signed.

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<220>
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gene of Agrobacterium T-DNA.

<220>
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<223> barnase: region coding for barnase

<220>
<221> misc_feature
<222> Complement((2625)..(4313))
<223> PE1: promoter region of E1 gene of rice

<220>
<221> misc_feature
<222> (4336)..(5170)
<223> P35S: 35S promoter region of Cauliflower Mosaic
Virus

<220>
<221> misc_feature
<222> Complement((5711)..(6262))
<223> bar: region coding for phosphinotricin
acetyltransferase

<220>
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<222> (6263)..(6496)
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T-DNA

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<220>
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<220>
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<222> (1)..(1601)
<223> promoter region of T72 gene of rice

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<212> DNA
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<220>
<223> Description of Artificial Sequence: plasmid pVE136

<220>
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<222> Complement((425)..(687))
<223> 3' nos: 3' untranslated region containing the
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gene of Agrobacterium T-DNA

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<222> Complement((803)..(1138))
<223> barnase: region coding for barnase

<220>
<221> misc_feature
<222> Complement((1138)..(2317))

<223> PCa55: stamen-specific promoter from corn gene CA55

<220>
<221> misc_feature
<222> (2355)..(3187)
<223> p35S: 35S promoter region of Cauliflower mosaic virus

<220>
<221> misc_feature
<222> (3188)..(3739)
<223> bar: region coding for phosphinoacetyl transferase

<220>
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<222> (3757)..(4017)
<223> 3' nos: 3' untranslated region containing the polyadenylation of the nopaline synthase gene of Agrobacterium T-DNA

<220>
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<223> region with unknown sequence (may contain up to 15 nucleotides)

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